PI: **RAFOLS, JOSE A**	Title: Assessing Polypathology after Traumatic Brain Injury	
Received: 06/03/2010	FOA: PA10-067	Council: 01/2011
Competition ID: ADOBE-FORMS-B	FOA Title: RESEARCH PROJECT GRANT (PARENT R01)	
1 R01 NS073603-01	Dual:	Accession Number: 3299643
IPF: 9110501	Organization: WAYNE STATE UNIVERSITY	
Former Number:	Department: Anatomy and cell Biology	
IRG/SRG: BINP	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 250,000 Year 2: 250,000 Year 3: 250,000 Year 4: 250,000 Year 5: 250,000	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: N Early Stage Investigator: N

Senior/Key Personnel:	*Organization:*	*Role Category:*
Jose Rafols PhD	Wayne State University	PD/PI
Mihir Bagchi PhD	Wayne State University	Faculty
Christian Kreipke PhD	Wayne State University	Faculty
Donald Kuhn PhD	Wayne State University	Faculty
Ewart Haacke PhD	Wayne State University	Faculty

OMB Number: 4040-0001
Expiration Date: 06/30/2011

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. * TYPE OF SUBMISSION

☐ Pre-application ☒ Application ☐ Changed/Corrected Application

4. a. Federal Identifier

b. Agency Routing Identifier

2. DATE SUBMITTED: 06/05/2010

Applicant Identifier

5. APPLICANT INFORMATION

*** Organizational DUNS:** 001962224

* Legal Name: Wayne State University

Department: Sponsored Program Admin. Division: Research

* Street1: 5057 Woodward Ave.

Street2: 13th Floor, Ste. 13202

* City: Detroit County / Parish: Wayne

* State: MI: Michigan Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 48202-4050

Person to be contacted on matters involving this application

Prefix: Ms. * First Name: Lisa Middle Name: M.

* Last Name: Ellis Suffix:

* Phone Number: 313-577-9120 Fax Number: 313-577-5055

Email: ak5050@wayne.edu

6. * EMPLOYER IDENTIFICATION *(EIN) or (TIN)*: 38-6028429

7. * TYPE OF APPLICANT: H: Public/State Controlled Institution of Higher Education

Other (Specify):

Small Business Organization Type ☐ Women Owned ☐ Socially and Economically Disadvantaged

8. * TYPE OF APPLICATION:

☒ New ☐ Resubmission

☐ Renewal ☐ Continuation ☐ Revision

If Revision, mark appropriate box(es).

☐ A. Increase Award ☐ B. Decrease Award ☐ C. Increase Duration ☐ D. Decrease Duration

☐ E. Other (specify):

* Is this application being submitted to other agencies? Yes ☐ No ☒ What other Agencies?

9. * NAME OF FEDERAL AGENCY: National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:

TITLE:

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:

Assessing Polypathology after Traumatic Brain Injury

12. PROPOSED PROJECT:

* Start Date: 04/01/2011 * Ending Date: 03/31/2016

*** 13. CONGRESSIONAL DISTRICT OF APPLICANT**: MI-013

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. * First Name: Jose Middle Name: A

* Last Name: Rafols Suffix: PhD

Position/Title: Professor

* Organization Name: Wayne State University

Department: Anatomy and cell Biology Division: Medicine

* Street1: 540 East Canfield

Street2: Room 9312

* City: Detroit County / Parish: Wayne

* State: MI: Michigan Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 48201-1928

* Phone Number: 313.993.4393 Fax Number: 313.577.3125

* Email: jrafols@med.wayne.edu

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested	1,868,527.00
b. Total Non-Federal Funds	0.00
c. Total Federal & Non-Federal Funds	1,868,527.00
d. Estimated Program Income	0.00

16. * IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES ☐ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ☒ PROGRAM IS NOT COVERED BY E.O. 12372; OR

☐ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

☒ * I agree

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or other Explanatory Documentation

Add Attachment | Delete Attachment | View Attachment

19. Authorized Representative

Prefix: Ms. * First Name: Lisa Middle Name: M.

* Last Name: Ellis Suffix:

* Position/Title: Grant & Contract Officer III

* Organization: Wayne State University

Department: Sponsored Program Admin. Division: Research

* Street1: 5057 Woodward

Street2: 13th Floor

* City: Detroit County / Parish: Wayne

* State: MI: Michigan Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 48202-4050

* Phone Number: 313-577-9120 Fax Number: 313-577-5055

* Email: ak5050@wayne.edu

*** Signature of Authorized Representative**

Lisa Ellis

*** Date Signed**

06/03/2010

20. Pre-application

Add Attachment | Delete Attachment | View Attachment

424 R&R and PHS-398 Specific Table Of Contents

OMB Number: 4040-0010
Expiration Date: 08/31/2011

Project/Performance Site Location(s)

Project/Performance Site Primary Location ☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Wayne State University

DUNS Number: 0019622240000

* Street1: 540 East Canfield

Street2: Rooms 9312, 9320, 9332

* City: Detroit County: WAYNE

* State: MI: Michigan

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 48201-1928 * Project/ Performance Site Congressional District: MI-013

Project/Performance Site Location 1 ☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:

DUNS Number:

* Street1:

Street2:

* City: County:

* State:

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: * Project/ Performance Site Congressional District:

Additional Location(s) Add Attachment Delete Attachment View Attachment

RESEARCH & RELATED Other Project Information

1. * Are Human Subjects Involved? ☐ Yes ☒ No

1.a If YES to Human Subjects

Is the Project Exempt from Federal regulations? ☐ Yes ☐ No

If yes, check appropriate exemption number. ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6

If no, is the IRB review Pending? ☐ Yes ☐ No

IRB Approval Date:

Human Subject Assurance Number:

2. * Are Vertebrate Animals Used? ☒ Yes ☐ No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ☒ Yes ☐ No

IACUC Approval Date:

Animal Welfare Assurance Number A3310-01

3. * Is proprietary/privileged information included in the application? ☐ Yes ☒ No

4.a. * Does this project have an actual or potential impact on the environment? ☐ Yes ☒ No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ☐ Yes ☐ No

4.d. If yes, please explain:

5. * Is the research performance site designated, or eligible to be designated, as a historic place? ☐ Yes ☒ No

5.a. If yes, please explain:

6. * Does this project involve activities outside of the United States or partnerships with international collaborators? ☐ Yes ☒ No

6.a. If yes, identify countries:

6.b. Optional Explanation:

7. * Project Summary/Abstract 1234-Abstract.pdf Add Attachment | Delete Attachment | View Attachment

8. * Project Narrative 1235-Project Narrative.pdf Add Attachment | Delete Attachment | View Attachment

9. Bibliography & References Cited 1236-References Cited.pdf Add Attachment | Delete Attachment | View Attachment

10. Facilities & Other Resources 1237-Resources.pdf Add Attachment | Delete Attachment | View Attachment

11. Equipment 1238-Equipment.pdf Add Attachment | Delete Attachment | View Attachment

12. Other Attachments Add Attachments | Delete Attachments | View Attachments

Abstract

Among multiple sequelae, TBI results in three major pathologies: 1) cerebral edema which leads to a critical rise in intracranial pressure, 2) diffuse axonal injury (DAI) which brings about disruption of neural circuits underlying cognitive and motoric behaviors, and 3) alterations in cerebral blood flow (CBF) that cause a persistent state of hypoperfusion and improper delivery of vital metabolites to neural tissue. While all three pathologies combine to cause substantial morbidity and mortality seen in the clinical setting, how these pathologies influence each other for final outcome is unclear. This laboratory has been successfully funded for the last decade to focus on the role of the endothelin (ET)-1 system in hypoperfusion following TBI. Recently, we have begun studies aimed at testing the effects of endothelin receptor A (ETrA) antagonists, which inhibit vasoconstriction (and hypoperfusion), as possible treatments for improving outcome following TBI. While experimental efficacy data is promising, the mechanism by which ETrA antagonists may exert their influence is not known. In the previous grant submission we continued to test the overall hypothesis that improving blood flow directly after TBI via ETrA antagonism can improve outcome after injury. However, as pointed out by several reviewers, hypoperfusion, alone, likely does not cause the deleterious effects of TBI. Thus, since the first submission we have designed a novel approach that aims to understand the mechanism by which ETrA antagonism may improve both histopathologic and behavioral outcome by focusing on polypathologies (in this case combined hypoperfusion and DAI). The central hypothesis of this grant is that: ***ETrA antagonism reduces the TBI-induced hypoperfusion and diminishes the extent of DAI, the combined effects from this intervention leading to improved histopathologic and behavioral outcomes***. Because the ability of ETrA antagonism to improve CBF has been the source of our previous funding, the present proposal focuses on the effect of hypoperfusion on DAI. Furthermore, since in parallel work in our lab aims to study the efficacy of ETrA antagonists in ameliorating behavioral deficits following TBI, this proposal is designed to augment his work with a mechanistic rationale for pursuing clinical trial with ETrA antagonists.

Project Narrative

Traumatic brain injury (TBI) is the leading cause of death and disability amongst our youth and children. Further, it has been named as the signature injury in the War on Terrorism that, upon return of our men and women fighting in Iraq and Afghanistan, is projected to cost millions in patient care and rehabilitation costs. While TBI results in three major pathologies, including diffuse axonal injury, brain edema, and hypoperfusion of the brain's parenchyma, this proposal investigates novel methods to increase blood flow and decrease extent of diffuse axonal injury after head trauma. In doing so, the experiments in this proposal are designed to yield results that can quickly be translated into the clinical setting, thus off-setting the current potentially dismal outcome following exposure to TBI.

Laboratory:

The laboratory consists of approximately 700 sq.ft. in which animal perfusion, tissue processing, sectioning and analysis can be performed. There is also an equipment room where the surgeries are conducted and which houses the trauma model. Dr. Rafols has additional laboratory of 700 sq.ft. that is used for behavioral testing.

Animal:

Facilities for the care and housing of experimental animals are available in the basement of Scott Hall. These resources are operated by the University Department of Laboratory Animal Resources. At WSU, all animals used for biomedical research at the medical center are housed in modern animal care facilities with excellent supervisory and veterinary support.

Computer:

Three Dell Dimension 8400 and One Toshiba Portege; 1 Epson Photo r100 printer.

Office:

The office facilities are located adjacent to the laboratory. The Principal Investigator and Co-investigator have their own office space fully equipped with computers and computer peripheria.

Equipment:

Three cryostats are available in the Department of Anatomy and Cell Biology; balance, ph meter, freezer, 2 refrigerators, dessicator, and equipment for ICC and Western blots are also available. Further equipment related to molecular biology is avialable to us from Dr. Kuhn's laboratory. All behavioral equipment including raidal arm maze, Morris water maze, treadmills, motor testing equipment are also currently available. Dr. Haacke will provide full access to MRI facilities.

OMB Number: 4040-0001
Expiration Date: 06/30/2011

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. * First Name: Jose Middle Name: A
* Last Name: Rafols Suffix: PhD
Position/Title: Professor Department: Anatomy and cell Biology
Organization Name: Wayne State University Division: Medicine
* Street1: 540 East Canfield
Street2: Room 9312
* City: Detroit County/ Parish: Wayne
* State: MI: Michigan Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 48201-1928
* Phone Number: 313.993.4393 Fax Number: 313.577.3125
* E-Mail: jrafols@med.wayne.edu

Credential, e.g., agency login: JOSERAFOLS

*** Project Role:** PD/PI **Other Project Role Category:**

Degree Type: PhD
Degree Year: 1969

***Attach Biographical Sketch** 1242-Biosketch_Rafols.pdf Add Attachment Delete Attachment View Attachment
Attach Current & Pending Support 1243-ongoing support_Rafols.p Add Attachment Delete Attachment View Attachment

PROFILE - Senior/Key Person 1

Prefix: Dr. * First Name: Mihir Middle Name:
* Last Name: Bagchi Suffix: PhD
Position/Title: Associate Professor Department: Anatomy and Cell Biology
Organization Name: Wayne State University Division: Medicine
* Street1: 540 East Canfield
Street2:
* City: Detroit County/ Parish: Wayne
* State: MI: Michigan Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 48201-1928
* Phone Number: 313.577.0574 Fax Number:
* E-Mail: mbagchi@med.wayne.edu

Credential, e.g., agency login: aa0839

*** Project Role:** Faculty **Other Project Role Category:**

Degree Type: PhD
Degree Year: 1969

***Attach Biographical Sketch** 1244-Biosketch_Bagchi.pdf Add Attachment Delete Attachment View Attachment
Attach Current & Pending Support Add Attachment Delete Attachment View Attachment

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 2

Prefix: Dr. * First Name: Christian Middle Name: William
* Last Name: Kreipke Suffix: PhD
Position/Title: Assistant Professor Department: Anatomy and Cell Biology
Organization Name: Wayne State University Division: Medicine
* Street1: 540 East Canfield
Street2:
* City: Detroit County/ Parish: Wayne
* State: MI: Michigan Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 48201-1928
* Phone Number: 313.577.1049 Fax Number: 313.577.3125
* E-Mail: ckreipke@med.wayne.edu

Credential, e.g., agency login: aa5930

*** Project Role:** Faculty **Other Project Role Category:**

Degree Type: PhD
Degree Year: 2004

***Attach Biographical Sketch**	1245-Biosketch_Kreipke.pdf	Add Attachment	Delete Attachment	View Attachment
Attach Current & Pending Support	1246-ongoing support_kreipke	Add Attachment	Delete Attachment	View Attachment

PROFILE - Senior/Key Person 3

Prefix: Dr. * First Name: Donald Middle Name: M
* Last Name: Kuhn Suffix: PhD
Position/Title: Professor Department: Psychiatry
Organization Name: Wayne State University Division: Medicine
* Street1: 540 East Canfield
Street2:
* City: Detroit County/ Parish: Wayne
* State: MI: Michigan Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 48201-1928
* Phone Number: 313.576.4458 Fax Number:
* E-Mail: donald.kuhn@wayne.edu

Credential, e.g., agency login: aa3071

*** Project Role:** Faculty **Other Project Role Category:**

Degree Type: PhD
Degree Year: 1976

***Attach Biographical Sketch**	1247-Biosketch_Kuhn.pdf	Add Attachment	Delete Attachment	View Attachment
Attach Current & Pending Support	1248-Ongoing support Kuhn.pdf	Add Attachment	Delete Attachment	View Attachment

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 4

Prefix: Dr. * First Name: Ewart Middle Name: Mark
* Last Name: Haacke Suffix: PhD
Position/Title: Professor Department: Radiology
Organization Name: Wayne State University Division: Medicine
* Street1: 540 East Canfield
Street2:
* City: Detroit County/ Parish: Wayne
* State: MI: Michigan Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 48201-1928
* Phone Number: 313.577.0574 Fax Number:
* E-Mail: nmrimaging@aol.com
Credential, e.g., agency login: ak5444
*** Project Role:** Faculty **Other Project Role Category:**
Degree Type: PhD
Degree Year: 1978

***Attach Biographical Sketch**	1249-Biosketch_Haacke.pdf	Add Attachment	Delete Attachment	View Attachment
Attach Current & Pending Support		Add Attachment	Delete Attachment	View Attachment

Principal Investigator/Program Director (Last, First, Middle): Rafols, Jose, A.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Jose A Rafols	POSITION TITLE Professor
eRA COMMONS USER NAME JOSERAFOLS	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Illinois Benedictine, Lisle, IL	B.S.	1965	Biology
University of Kansas, Kansas City, KS	Ph.D.	1969	Anatomy
S. Ramon Y Cajal Institute, CSIC, Madrid, Spain	Post Doc	1970	Neuroanatomy

A. Personal Statement

Traumatic brain injury (TBI) is the leading cause of death and disability among children and young adults. TBI results in 3 major pathologies: 1. Cerebral edema which leads to elevated ICP, 2. Diffuse axonal injury which brings about disruption of neural circuits underlying cognitive behavior, and 3. Alterations in the brain's microcirculation that cause persistent hypoperfusion and improper delivery of vital metabolites to neural tissue. While clinical trials aimed at the first two pathologies have been developed, to date none has addressed the third pathology, hypoperfusion following TBI. The present proposal uses a novel approach to understanding how ETrA antagonists may be effective in treating TBI by centering on co-pathologies (e.g., DAI and hypoperfusion). Therefore, this proposal is a logical extension of my previous work using BQ-123, a non clinically relevant ETrA antagonist, that has translational potential.

B. Positions and Honors

Positions and Employment

1969-1970	Instructor, Dept. of Anatomy/Cell Biology, Wayne State University, School of Medicine
1970	NIH Postdoctoral trainee at S. Ramon Y Cajal Institute, CSIC, Madrid, Spain
1971-1973	Asst. Professor, Dept. of Anatomy/Cell Biology, Wayne State University, School of Medicine
1973-1989	Assoc. Professor, Dept. of Anatomy/Cell Biology, Wayne State University, School of Medicine
1989-present	Professor, Dept. of Anatomy/Cell Biology, Wayne State University, School of Medicine
1994-present	Dir., Morphology and Imaging Core, Neurotrauma Center, Wayne State University, School of Medicine

Honors

DHHS/PHS/NIH Study Section Member (full member), Neurological Disorder Program Project Review A Committee (NSP-term) 7/1/90-6/30/94.
Chairman, Site visit, The Johns Hopkins University, Baltimore, MD; "Disorders of aging neuro-transmitter systems and neurotrophins", December 15-17, 1991.
Member, National Institutes of Health Reviewers Reserve (NRR), for term 7/1/94-6/30/98.
Member, American Heart Association National Study Committee, Brain Review Committee, for term 7/96-6/99.

C. Peer-reviewed publications

1. Kreipke CW, Morgan N, Petrov T, Rafols J. 2006. Calponin and caldesmon cellular domains in reacting microvessels following traumatic brain injury. Microvascular Research 71:197-204.
2. Shen Y, Kou Z, Kreipke CW, Petrov T, Hu J, Haacke EM. 2007. In vivo measurement of tissue damage, oxygen saturation changes and blood flow changes after experimental traumatic brain injury in rats using susceptibility-weighted imaging. Magn Reson Imaging 25:219-227.
3. Kreipke CW, Morgan R, Petrov T, Rafols JA. 2007. Subcellular Redistribution of Calponin Underlies Sustained Vascular Contractility Following Traumatic Brain Injury. Neurological Research 29:604-609.
4. Kallukuri S, Kreipke CW, Rossi N., Rafols JA, Petrov T. 2007. Spatial alterations in endothelin receptor expression are temporally associated with the altered microcirculation after brain trauma. Neurological Research 29:362-368.
5. Kreipke CW, Morgan R, Roberts G, Bagchi M, Rafols JA. 2007. Calponin phosphorylation in cerebral cortex microvessels mediates sustained vasoconstriction after brain trauma. Neurological Research 29:369-374.
6. Kreipke CW, Petrov T, Rafols JA. 2007. Endothelin A receptor antagonism blocks calponin phosphorylation following brain trauma. J Cereb Blood Flow and Metab, 26, S191.
7. Kreipke CW, Schafer PC, Rafols JA. 2008. Endothelin receptor A antagonism ameliorates hypoperfusion and enhances cognitive outcome following traumatic brain injury. Brain Injury 22:S43.
8. Rafols JA, Kreipke CW, Kallakuri S. 2008. Upregulation of endothelin-1 receptors in neurons and brain microvessels coincides temporally with a dysfunctional microcirculation after traumatic brain injury. Brain Injury 22:S44.
9. Kreipke CW, Rafols JA. 2009. Calponin control of cerebrovascular reactivity: Therapeutic implications in brain trauma. J Cell Mol Med 13(2):262-9.
10. Ding JY, Kreipke CW, Speirs S, Schafer PC, Schafer S, Rafols JA. 2009. Hypoxia inducible factor-1□ signaling in aquaporin upregulation after traumatic brain injury. Neuros Lett. 453(1):68-72.
11. Ding JY, Kreipke CW, Speirs S, Schafer PC, Schafer S, Rafols JA. 2009. Synapse Loss Regulated by Matrix Metalloproteinases in Traumatic Brain Injury Is Associated with Hypoxia-Inducible Factor-1α Expression. Brain Research 1268:125-34.
12. Kreipke CW, Schafer PC, Rossi NF, Rafols JA. 2009 (Epub ahead of press). Differential affects of Endothelin receptor-A and B antagonism on hypoperfusion following traumatic brain injury (TBI). Neurological Research.
13. Kallakuri S, Kreipke CW, Schafer PC, Schafer SM, Rafols JA. (in press) Brain cellular localization of endothelin receptor A and B in a rodent model of diffuse brain injury. Neuroscience.
14. Kallakuri S, Kreipke CW, Schafer PC, Schafer SM, Rafols JA. 2010 Brain cellular localization of endothelin receptor A and B in a rodent model of diffuse brain injury. Neuroscience. Epub ahead of press.
15. Dore-Duffy P, Ding Y, Zhan P, Schafer S, Fronczak M, Rafols JA, Kreipke CW. (in press) Endothelin receptor expression following ETrA antagonist treatment. Neurological Reseach.

D. Research Support

Ongoing Research Support

R01 NS064976-A2 Kreipke (PI) 11/01/09-10/31/14
NIH_NINDS
Role: CO-I
"Molecular Mechanisms of Enhanced Contractility following Traumatic Brain Injury: towards a clinical trial" (Investigates the mechanism by which endothelin receptor antagonists may be useful in the treatment of cognitive deficits following TBI).

VARR&D 1I01RX000224-01 Kreipke (PI) 11/01/09-10/31/12
Role: CO-I

Principal Investigator/Program Director (Last, First, Middle): Rafols, Jose, A.

"Poly-trauma following brain injury: towards a combinatorial therapy" (Investigates the effects of multiple pathologies associated with traumatic brain injury on histopathological and behavioral outcome).

VA RR & D Award Rossi/Kreipke (PI) 04/01/08-12/31/11
VA Rehabilitation
Role: CO-I
"Conditioning, microvascular tone & rehabilitation post brain trauma" (Investigates the role of exercise in the control of microcirculation in a rat model of traumatic brain injury).

Principal Investigator/Program Director (Last, First, Middle): Rafols, Jose. A.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME MIhir Bagchi	POSITION TITLE Associate Professor
eRA COMMONS USER NAME Aa0839	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Bihar University, India	B.S.	1959	Biology, Chemistry
Rachi University, India	M.A.	1962	Zoology
University of Vermont	Ph.D.	1969	Zoology

A. Personal Statement

Traumatic brain injury (TBI) is the leading cause of death and disability among children and young adults. TBI results in 3 major pathologies: 1. Cerebral edema which leads to elevated ICP, 2. Diffuse axonal injury which brings about disruption of neural circuits underlying cognitive behavior, and 3. Alterations in the brain's microcirculation that cause persistent hypoperfusion and improper delivery of vital metabolites to neural tissue. While clinical trials aimed at the first two pathologies have been developed, to date none has addressed the third pathology, hypoperfusion following TBI. I have extensive experience in molecular biology and will help in any way that I can, particularly in terms of biochemical assays.

B. Positions and Honors

Positions and employment

1966-1968	Graduate Assistant, University of Vermont, Burlington, VT.
1969-1972	Postdoctoral Research Associate, Oakland University, Rochester, MI.
1972-1973	Instructor, Dept. of Biological Science, Oakland University, Rochester, MI.
1973-1975	NIH Special Fellow, Kresge Eye Institute, Detroit, MI.
1975-1981	Assistant Professor, Wayne State University, Anatomy/Cell Biology, Detroit, MI.

C. Peer-reviewed publications

M. Bagchi, A. Roher, A. Banerjee, R. Barrett, L. Hazlett, T. Kasunic, and H. Maisel. Identification of a ubiquitin like protein in the mammalian vitreous humor. J. Cell Biochem. 61:26-31, 1996.
M. Bagchi, S. Anasari, M. Katar and H. Maisel. Non-chromatin nuclear proteins of mammalian lens epithelial cells. J. Cell Biochem. 64:644-650, 1997.
M. Bagchi, S. Ansari, D.M. Lindenmuth, A. VanWijnen, J. Lian, J. Stein and G. Stein. Nuclear matrix associated DNA binding proteins of ocular lens epithelial cells. Molecular Biology Reports. 25:13-19, 1998.
M. Bagchi, M. Katar and H. Maisel. A heat shock factor-like protein in the nucleus of tissue cultured lens epithelial cells. J. Cell Biochem. 80:382-387, 2001.
M. Bagchi, M. Ireland, M. Katar and H. Maisel. Heat shock proteins of the chicken lens. J. Cell Biochem. 82:409-414, 2001.
M. Bagchi, M. Katar and H. Maisel. Heat shock protein of adult and embryonic human ocular lenses. J. Cell Biochem. 84:278-284, 2002.
M. Bagchi and H. Maisel. Effect of exogenous stress on the tissue cultured mouse lens epithelial cell. J. Cell Biochem. 86:302-306, 2002.
M. Bagchi, M. Katar, J. Lewis and H. Maisel. Associated proteins of lens adherence junction. J. Cell Biochem. 86:700-703, 2002.
M. Bagchi, M. Katar, K. Lo, H. Maisel. Paralamimn of the chicken lens. J. Cell Biochem. 89:917-921, 2003.
M. Bagchi, M. Katar, W.-K. Lo, R. Yost, C. Hill and H. Maisel. ERM proteins of the lens. J. Cell Biochem. 92:626-630, 2004.
K.R. Badri, S. Modem, H. Gerard, I. Khan, M. Bagchi, A.R. Hudson, and T.R. Reddy. Regulation of sam 68 activity by small heat shock protein 22. J. Cell Biochem. J. Cell Bio. 99:1353-1362, 2006.
M. Bagchi, T. Petrov and H. Maisel. Lamins of ocular lens epithelial cells. J. Cell Biochem.150:923-928, 2007.
M. Bagchi, T.R. Reddy, R. Skoff, S. Modem, D.A, Bessert, and H. Maisel. Effect of thermal stress on the early and late passaged mouse lens epithelial cells. J. Cell Biochem. 102:1036-1042, 2007.
C.W. Kreipke, R. Morgan, G. Roberts, M. Bagchi and J. Rafols. Calponin phosphorylation in cerebral cortex microvessel mediates sustained vasoconstriction after traumatic brain injury. Neurol. Res. 29:369-374, 2007.
R. Morgan, C.W. Kreipke, G. Roberts, M. Bagchi and J. Rafols. Neurovascularization following traumatic brain injury, possible evidence for both angiogenesis and vasculogenesis. Neurol. Res. 29:375-381, 2007.

D. Research Support

NONE

Principal Investigator/Program Director (Last, First, Middle): Rafols, Jose. A.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Christian W. Kreipke	POSITION TITLE Assistant Professor
eRA COMMONS USER NAME aa5930	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Wayne State University	B.A.	1995-1999	Anthropology
Wayne State University	M.A.	1999-2000	Medical Anthropology
Wayne State University, School of Medicine	Ph.D.	2000-2004	Neuroscience
Wayne State University, School of Medicine	Postdoc	2004-2007	TBI

A. Personal Statement

Traumatic brain injury (TBI) is the leading cause of death and disability among children and young adults. TBI results in 3 major pathologies: 1. Cerebral edema which leads to elevated ICP, 2. Diffuse axonal injury which brings about disruption of neural circuits underlying cognitive behavior, and 3. Alterations in the brain's microcirculation that cause persistent hypoperfusion and improper delivery of vital metabolites to neural tissue. While clinical trials aimed at the first two pathologies have been developed, to date none has addressed the third pathology, hypoperfusion following TBI. The present proposal uses a novel antagonist of endothelin-1 receptor A (ETrA), Clazosentan, to improve CBF and ultimately cognition. My laboratory has been primarily interested in how different endothelin receptor antagonists impact both CBF and behavioral outcome following TBI. This work includes published proof of concept data that supports the use of ETrA antagonists as a means to decrease the extent of hypoperfusion following TBI. Therefore, this proposal is a logical extension of my work which could further the mechanistic rationale for using ETrA antagonists as clinical treatments for TBII.

B. Positions and Honors

Positions and employment

01/97-05/97 Wayne State University, School of Medicine and Hutzel Hospital, Research Assistant, Bone Densitometry/Osteoporosis Project

09/97-09/99 Wayne State University, Institute for Information and Technology, Research Assistant, HIV/AIDS in Detroit Project

09/99-05/00 Wayne State University, Graduate Teaching Assistant, Department of Anthropology

05/00-09/00 Wayne State University, Adjunct Instructor, Department of Anthropology

09/00-08/04 Wayne State University, School of Medicine, Pre-Doctoral Research Assistant, National Institute of Drug Abuse T32 Training Grant

08/04-04/08 Wayne State University, School of Medicine, Research Associate, Dept. Anatomy and Cell Biology, Traumatic Brain Injury

04/08-present Wayne State University, School of Medicine, Research Scientist, Dept. Anatomy and Cell Biology

Principal Investigator/Program Director (Last, First, Middle): Rafols, Jose. A.

Other Experience and Professional Memberships

05/99-present	Member, Phi Beta Kappa
02/00-present	Member, Society for Applied Anthropology
02/00-present	Member, Society for Medical Anthropology
05/01-present	Member, Sigma Xi
05/01-present	Member, New York Academy of Sciences
03/01-03/02	Society for Neuroscience Brain Awareness Week Committee, Wayne State University, Chair
05/02-present	Member, Society for Neuroscience
05/02-05/04	Michigan Society for Neuroscience, Student Counselor
05/03	Michigan Society for Neuroscience Chapter Meeting coordinator
11/04-08/07	Sigma Xi, Wayne State Chapter, Executive Board Member
02/05-08/07	Wayne State Alumni Communications Committee, Committee Member
05/06-08/07	Sigma Xi, National, Associate Director, NorthCentral Region
03/07-present	Member, International Society for Cerebral Blood Flow and Metabolism
02/07-present	Chairman of the Board, Southfield Oncology Institute
08/07-present	Sigma Xi, National, Acting Director, NorthCentral Region
02/08-present	Member of The Royal Society of Chemistry

Honors

2002 Dean Thomas Asselin, M.D. Endowed Prize for Excellence in Psychiatry and Behavioral Neuroscience Research (Wayne State University School of Medicine)

2003 1st Place, Society for Neuroscience, MI Chapter, Poster Award

2006 Service Award For 2006 Sigma Xi National Conference

2007 Travel Award, Brain '07, Society for Cerebral Blood Flow and Metabolism

2007 Young Investigators Award, Endothelin 10, Endothelin

2010 Travel Award, Winter Brain

C. Peer-reviewed publications (from 32 selected works)

1. Kreipke CW, Morgan N, Petrov T, Rafols J. 2006. Calponin and caldesmon cellular domains in reacting microvessels following traumatic brain injury. Microvascular Research 71:197-204.
2. Shen Y, Kou Z, Kreipke CW, Petrov T, Hu J, Haacke EM. 2007. In vivo measurement of tissue damage, oxygen saturation changes and blood flow changes after experimental traumatic brain injury in rats using susceptibility-weighted imaging. Magn Reson Imaging 25:219-227.
3. Kreipke CW, Morgan R, Petrov T, Rafols JA. 2007. Subcellular Redistribution of Calponin Underlies Sustained Vascular Contractility Following Traumatic Brain Injury. Neurological Research 29:604-609.
4. Kallukuri S, Kreipke CW, Rossi N., Rafols JA, Petrov T. 2007. Spatial alterations in endothelin receptor expression are temporally associated with the altered microcirculation after brain trauma. Neurological Research 29:362-368.
5. Kreipke CW, Morgan R, Roberts G, Bagchi M, Rafols JA. 2007. Calponin phosphorylation in cerebral cortex microvessels mediates sustained vasoconstriction after brain trauma. Neurological Research 29:369-374.

6. Kreipke CW, Petrov T, Rafols JA. 2007. Endothelin A receptor antagonism blocks calponin phosphorylation following brain trauma. J Cereb Blood Flow and Metab, 26, S191.
7. Kreipke CW, Schafer PC, Rafols JA. 2008. Endothelin receptor A antagonism ameliorates hypoperfusion and enhances cognitive outcome following traumatic brain injury. Brain Injury 22:S43.
8. Rafols JA, Kreipke CW, Kallakuri S. 2008. Upregulation of endothelin-1 receptors in neurons and brain microvessels coincides temporally with a dysfunctional microcirculation after traumatic brain injury. Brain Injury 22:S44.
9. Kreipke CW, Rafols JA. 2009. Calponin control of cerebrovascular reactivity: Therapeutic implications in brain trauma. J Cell Mol Med 13(2):262-9.
10. Ding JY, Kreipke CW, Speirs S, Schafer PC, Schafer S, Rafols JA. 2009. Hypoxia inducible factor-1□ signaling in aquaporin upregulation after traumatic brain injury. Neuros Lett. 453(1):68-72.
11. Ding JY, Kreipke CW, Speirs S, Schafer PC, Schafer S, Rafols JA. 2009. Synapse Loss Regulated by Matrix Metalloproteinases in Traumatic Brain Injury Is Associated with Hypoxia-Inducible Factor-1α Expression. Brain Research 1268:125-34.
12. Kreipke CW, Schafer PC, Rossi NF, Rafols JA. 2009 (Epub ahead of press). Differential affects of Endothelin receptor-A and B antagonism on hypoperfusion following traumatic brain injury (TBI). Neurological Research.
13. Kallakuri S, Kreipke CW, Schafer PC, Schafer SM, Rafols JA. (in press) Brain cellular localization of endothelin receptor A and B in a rodent model of diffuse brain injury. Neuroscience.
14. Kreipke CW, Schafer PC, Schafer S, Pirooz R, Rafols JA (in press) Endothelin receptors A and B are expressed in distinct cellular compartments of rat hippocampus following global ischemia: An immunocytochemical study. Neurological Research.
15. Armstead W, Kreipke CW. (in press) Endothelin-1 is upregulated after traumatic brain injury: A cross-species, cross-model analysis. Neurological Research

D. Research Support

Ongoing Research Support

R01 NS064976-A2 Kreipke (PI) 11/01/09-10/31/14
NIH_NINDS
Role: PI
"Molecular Mechanisms of Enhanced Contractility following Traumatic Brain Injury: towards a clinical trial" (Investigates the mechanism by which endothelin receptor antagonists may be useful in the treatment of cognitive deficits following TBI).

VARR&D 1I01RX000224-01 Kreipke (PI) 11/01/09-10/31/12
Role: PI
"Poly-trauma following brain injury: towards a combinatorial therapy" (Investigates the effects of multiple pathologies associated with traumatic brain injury on histopathological and behavioral outcome).

VA RR & D Award Rossi/Kreipke (PI) 04/01/08-12/31/11
VA Rehabilitation
Role: CO-PI
"Conditioning, microvascular tone & rehabilitation post brain trauma" (Investigates the role of exercise in the control of microcirculation in a rat model of traumatic brain injury).

Principal Investigator/Program Director (Last, First, Middle): Rafols, Jose, A.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Donald M. Kuhn	Professor
eRA COMMONS USER NAME	
aa3071	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Presbyterian College	BS	1972	Biopsychology
University of South Carolina	PhD	1976	Behavioral Pharmacology
Princeton University	Postdoc	1976-1977	Electrophysiology
National Institutes of Health	Postdoc	1977-1983	Biochemical Pharmacology

A. Personal Statement

Traumatic brain injury (TBI) is the leading cause of death and disability among children and young adults. TBI results in 3 major pathologies: 1. Cerebral edema which leads to elevated ICP, 2. Diffuse axonal injury which brings about disruption of neural circuits underlying cognitive behavior, and 3. Alterations in the brain's microcirculation that cause persistent hypoperfusion and improper delivery of vital metabolites to neural tissue. While clinical trials aimed at the first two pathologies have been developed, to date none has addressed the third pathology, hypoperfusion following TBI. The present proposal uses a novel antagonist of endothelin-1 receptor A (ETrA), Clazosentan, to improve CBF and ultimately cognition. My laboratory has a long history of performing pharmacological studies and therefore I will aide Dr. Rafols with his design of dosing and dosing regimes.

B. Positions and Honors

Positions and Employment

1983-1986-Chief, Section on Biochemical Pharmacology, National Heart Lung & Blood Institute, NIH
1985-1986-Alexander von Humboldt Fellow, Department of Neurochemistry, Goethe University, Frankfurt, Germany
1987-present- Professor, Department of Psychiatry and Behavioral Neurosciences, Center for Molecular Medicine and Genetics, and Institute for Chemical Toxicology, Wayne State University School of Medicine
1993-1994-Visiting Professor, Dept. Molecular Genetics and HHMI, Univ. Texas Southwestern Medical Center, Dallas, Texas (Sabbatical leave in Dr. T. Sudhof's lab)
1998-present- Research Career Scientist, John D. Dingell VA Medical Center, Detroit, MI

Other Experience and Professional Memberships

1994-1998 Member, NIDA-C (now NMB) Scientific Review Subcommittee
1998-2002 Member, MDCN-4 Scientific Review Subcommittee
1999- Member, Editorial Board Journal of Neurochemistry
1999- Ad hoc reviewer for MDCN-3, IFCN-7, Neurological Sciences & Disorders B, NIDA Cebra Program, and numerous SEPs for NIDA, NINDS, and NIMH
2001- National Scientific Advisory Council, American Federation for Aging Research
2004- Member, Neurobiology A Merit Review Subcommittee, Dept. Veterans Affairs
2006- Member, NMB Scientific Review Subcommittee

Honors

Principal Investigator/Program Director (Last, First, Middle): Kreipke, Christian, W.

1985- Fellow, Alexander von Humboldt Foundation

C. Selected peer-reviewed publications (in chronological order)

(Publications selected more than 135 peer-reviewed publications and book chapters)

Wolf, W.A. and **Kuhn, D.M.** Molecular pharmacology of the neuronal serotonin transporter: Role of essential sulfhydryl groups in ligand binding and transport. J. Biol. Chem. 267, 20820-20825, 1992.

Kuhn, D.M. and Geddes, T.J. Peroxynitrite inactivates tryptophan hydroxylase via sulfhydryl oxidation: Coincident nitration of enzyme tyrosyl residues has minimal impact on catalytic activity. J. Biol. Chem. 274, 29726-29732, 1999.

Anastasiadis, P.Z., Jiang, H., Bezin, L., **Kuhn, D.M.**, and Levine, R.A. Tetrahydrobiopterin enhances apoptotic cell death following withdrawal of trophic support. J. Biol. Chem. 276, 9050-9058, 2001.

Kuhn, D.M. Dopamine and Its Modulation of Drug-Induced Neuronal Damage, E. Massaro (Ed.). In: Handbook of Neurotoxicology, Volume 2, Drugs of Abuse, Humana Press, pp 175-197, 2002.

Kuhn, D.M., Sadidi, M., Lu, X., Kreipke, C., Geddes, T., Borges, C., and Watson, J.T. Peroxynitrite-induced nitration of tyrosine hydroxylase: Identification of tyrosines 423, 428, and 432 as sites of modification by MALDI-TOF mass spectrometry and tyrosine-scanning mutagenesis. J. Biol. Chem., 277, 14336-14342, 2002.

Kuhn, D.M. and Geddes, T.J. Reduced nicotinamide nucleotides prevent nitration of tyrosine hydroxylase by peroxynitrite. Brain Research, 933, 85-89, 2002.

Thomas, D.M., Dowgiert, J., Geddes, T.J., Verbeem, D., Liu, X., and **Kuhn, D.M.** Microglial activation is a pharmacologically specific marker for the neurotoxic amphetamines. Neurosci. Lett., 367, 349-354, 2004.

Thomas, D.M. and **Kuhn, D.M.** Attenuated microglial activation mediates tolerance to the neurotoxic effects of methamphetamine. J. Neurochem., 92, 790-797, 2005.

Thomas, D.M., Francescutti-Verbeem, D.M., and **Kuhn, D.M.** Gene expression profile of activated microglia under conditions associated with dopamine neuronal damage. FASEB J (FJ Express Summary), 20, 515-517, 2006.

Kuhn, D.M., Sakowski, S.A., Geddes, T.J., Wilkerson, C., and Haycock, J.W. Phosphorylation and activation of tryptophan hydroxylase 2: Identification of serine-19 as the substrate site for calcium-dependent protein kinase II. J. Neurochem., 103, 1567-1573, 2007.

Thomas, D.M., Francescutti-Verbeem, D.M., and **Kuhn, D.M.** The newly synthesized pool of dopamine determines the severity of methamphetamine-induced neurotoxicity. J. Neurochem., 605-616, 2008.

Kuhn, D.M., Francescutti-Verbeem, D.M., and Thomas, D.M. Dopamine disposition in the presynaptic process regulates the severity of methamphetamine-induced neurotoxicity. Ann. N.Y. Acad. Sci., in press, 2008.

Kuhn, D.M., Francescutti-Verbeem, D.M., and Thomas, D.M. Dopamine disposition in the presynaptic process regulates the severity of methamphetamine-induced neurotoxicity. Ann. N.Y. Acad. Sci., 1139, 118-126, 2008.

Thomas, D.M., Francescutti-Verbeem, D.M., and **Kuhn, D.M.** Increases in cytoplasmic dopamine compromise the normal resistance of the nucleus accumbens to methamphetamine neurotoxicity. J. Neurochem., 109, 1745-1755, 2009.

Kreipke, C.W., Schafer, P.C., Schafer, S.M., Pirooz, R., Angoa-Perez, M., Rafols, J.A. and **Kuhn, D.M.** Validation of a mouse acceleration-impact model of traumatic brain injury. J. Neurotrauma, in press, 2009.

D. Research Support

Ongoing (Active) Research Support

NIH/NIDA 5 R01 DA10756 04/10/07-04/09/12
Neurotoxic Amphetamines, Radicals, and 5HT Neurons
The major goal of the study is to determine the mechanisms by which neurotoxic amphetamine-derived reactive oxygen and nitrogen species alter function of dopamine and serotonin neurons through their effects on important phenotypic marker proteins in these neuronal elements.
Role: PI

NIH/NIDA 1 RO1 DA017327 04/01/05 – 03/30/10

Principal Investigator/Program Director (Last, First, Middle): Kreipke, Christian, W.

Methamphetamine Neurotoxicity and Microglial Activation
The goal of this project is to elucidate the role of microglia in the neurotoxic effects associated with methamphetamine and other neurotoxic amphetamines.
Role: PI

Department of Veterans Affairs Merit Award 03/15/07-03/14/11
Brain Injury by Blast Overpressure: Role of Microglial Activation
The goal of this project is to characterize microglial involvement in brain damage caused by blast overpressure. We have developed a model of blast overpressure, a form of traumatic brain injury, that allows testing of cultured cells and brain slices.
Role: PI

R01 NS064976-A2 Kreipke (PI) 11/01/09-10/31/14
NIH_NINDS
"Molecular Mechanisms of Enhanced Contractility following Traumatic Brain Injury: towards a clinical trial" (Investigates the mechanism by which endothelin receptor antagonists may be useful in the treatment of cognitive deficits following TBI).
Role: Co-I

VARR&D 1I01RX000224-01 Kreipke (PI) 11/01/09-10/31/12
"Poly-trauma following brain injury: towards a combinatorial therapy" (Investigates the effects of multiple pathologies associated with traumatic brain injury on histopathological and behavioral outcome).
Role: Co-I

Projects completed in the past 3 years

NIH/NIDA 1 K05 DA14692 10/05/02-12/04/07
Molecular Biology of Drug Abuse
This is a senior scientist career development award.
Role: PI

NIH/NIDA 1 T32 DA07310 07/01/00-06/30/06
Neuroscience Training in Drug Abuse
This is a training grant that supports two predoctoral and two postdoctoral fellows. This training program is in hiatus temporarily. Our department experienced some significant changes in faculty re-assignment to other academic units, and several other key investigators on the T32 have left Wayne State. Therefore, we are re-configuring this training program as the Translational Neuroscience Program to reflect more accurately the current mentoring and research expertise of our departmental faculty.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
E. Mark Haacke, PhD	Professor and Directors
eRA COMMONS USER NAME ak5444	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Toronto	B.S.	1973	Mathematics & Physics
University of Toronto	M.S.	1975	Theoretical Physics
University of Toronto	Ph.D.	1978	High Energy Physics

A. Personal Statement

Prof. Haacke has been focusing on traumatic brain injury projects for the last five years. He has been instrumental in evaluating TBI using modern advanced imaging technologies such as susceptibility weighted imaging for example. His background is in the development and application of new imaging methods such as SWI and diffusion tensor imaging (DTI). He was involved in the NINDS/NIH 2009 workshop on TBI and has been involved in the preparation of an imaging report to the community that is currently under review. Finally, Prof. Haacke has been collaborating with all personnel on this project for the last five years as well.

B. Positions and Honors.

Positions and Employment

1981-1983 **Research Geophysicist**, Gulf Research and Development, Pittsburgh, PA.
1983-1985 **Senior Research Scientist**, Picker International, Highland Heights, OH.
1985-1989 **Assistant Professor of Radiology and Physics**, Head, MR Physics and Basic Science. Case Western Reserve University, Cleveland, OH.
1989-1993 **Associate Professor**, Department of Radiology with appointments in Physics and Biomedical Engineering, Case Western Reserve University, Cleveland, OH.
1993-1999 **Professor of Radiology**, Director MR Imaging Research, Mallinckrodt Institute of Radiology, Washington University, St. Louis, MO.
1999-Present **Director**, The MRI Institute for Biomedical Research, Detroit, MI.
2002-Present **Professor of Radiology**, Wayne State University, Detroit, MI.
2002-Present **Director**, Wayne State University, Magnetic Resonance Imaging Facility, Detroit, MI.
2002-Present **Professor of Biomedical Engineering**, Wayne State University, Detroit, MI.
2002-Present **Adjunct Professor**, Loma Linda University, Loma Linda, CA.
2005-Present **Adjunct Professor**, Department of Electrical and Computer Engineering at McMaster University and the Brain-Body Institute at St Joseph's Healthcare in Hamilton, Ontario, Canada.

Other Experience and Professional Memberships

1983-1985 **Lecturer in Physics**, New course on MRI, Case Western Reserve University, Cleveland, OH.
1992-1992 **Associate Editor**, IEEE for Transactions on Medical Physics.
1992-1994 **Chairman**, Liaison Committee at the Society for Magnetic Resonance Imaging (SMRI).
1992-1994 **Co-founder**, Joint Merger Evaluation Committee for the Society for Magnetic Resonance Imaging (SMRI) / International Society for Magnetic Resonance in Medicine (ISMRM).
1993 **Vice-President**, Interim Board at the Society of Magnetic Resonance Imaging (SMRI).
1993-1994 **President**, Society of Magnetic Resonance Imaging (SMRI).
2007-Present **Assoc Chair**, School of Medicine, Dept of Biomedical Eng, Wayne State University, Detroit, MI.

Honors

1989 Sylvia Sorken Greenfield Award for the best paper in Medical Physics
1992 Fellow of the Society Award for the Society of Magnetic Resonance Imaging

1994 Silver Medal Award, Society of Magnetic Resonance
1997 Poster Award at the 14th Annual Meeting, European Society for Magnetic Resonance in Medicine and Biology. J.R. Reichenbach, E.M. Haacke, B.C.P. Lee, Ch. Przetak, W.A. Kaiser
1998 Marie-Sklodowska-Curie Prize for Visualization of Cerebral Venous Structures Using High Resolution MRI by J.R. Reichenbach, L.R. Schad, M. Essig, E.M. Haacke, W.A. Kaiser
1999 Awarded the Visiting Professorship as the Roentgen Professor of Physics in Wuerzburg.
2000 Poster Prize of the XXVI Congress of the European Society of Neuroradiology 2000. J.R. Reichenbach, L. Jonetz-Mentzel, C. Fitzek, H.-J. Mentzel, E.M. Haacke, W.A. Kaiser.
2002 Scientific Exhibition Award ECR 2002 Cum Laude. J.R. Reichenbach, C. Fitzek, L. Jonetz-Mentzel, D. Sauner, H.-J. Mentzel, E.M. Haacke, W.A. Kaiser. European Congress of Radiology
2004 Gold Medal Award, International Society of Magnetic Resonance in Medicine
2006 Wayne State University, Office of the Vice President for Research, Research Mentors Award Program for New Faculty for mentoring of Dr. Yu-Chung Norman Cheng
2006 RSNA Educational Exhibit Award LL-NR4709 entitled "Susceptibility Weighted Imaging (SWI) of the Brain: Pictorial Review of the Technique, Anatomy, and Pathology" T. Hirai, MD, Kumamoto JAPAN; M. Akter; M. Kitajima, MD; T. Okuda, MD; E.M. Haacke, PhD; Y. Yamashita, MD
2008 Best Abstract Award "Improving the detection of diffue axonal injury by complementary use of advanced MRI" at the 6th North American Brain Injury (NABIS) Annual Conference. Z. Kou, R. Benson, R. Gattu, M. Haacke. The abstract presented our breakthrough on a complementary use of SWI and DTI techniques for injury detection.
2009 Regional Scholarship for Asia "Imaging the Vessel Wall in Major Peripheral Arteries using Susceptibility Weighted Imaging: Visualizing Calcifications" at the 12th Annual Society of Cardiovascular Magnetic Resonance (SCMR). Qi Yang, Kuncheng Li, Jiangtao Liu, S. Barnes, Z. Wu, J. Neelavalli, J. Hu, E.M. Haacke.

C. Selected peer-reviewed publications. (Selected from 218 peer-reviewed publications)

Most relevant to the current application

1. E.M. Haacke, F.H. Bearden, J.R. Clayton and N.R. Linga. Reduction of MR Imaging Time by the Hybrid Fast Scan Technique. Radiology **1986**;158:521-529.
2. E.M. Haacke, C.L. Filleti, R. Gattu, C. Ciulla, A.Al-Bashir, K. Suryanarayanan, M. Li, Z. Latif, Z. DelProposto, V. Sehgal, T. Li, V. Torquato, R. Kanaparti, J. Jiang, J. Neelavalli. New Algorithm for Quantifying Vascular Changes in Dynamic Contrast-Enhanced MRI Independent of Absolute *T*1 Values. MRM **2007** – 58:463-472.
3. Hillman GG, Singh-Gupta V, Zhang H, Al-Bashir AK, Katkuri Y, Li M, Yunker CK, Patel A, Abrams J, Haacke EM. DCE-MRI of vascular changes induced by sunitinib in papillary renal cell carcinoma xenograft tumors. Neoplasia **2009** - 11;910-920. PMCID: PMC2735805.
4. Yu Y., Q. Jiang , Y. Miao, J. Li , H. Wang, S. Bao, C. Wu, X. Wang., J. Zhu, Y. Zhong, EM Haacke, J. Hu. "Quantitative analysis of clinical dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) to evaluate treatment response in human breast cancer" is conditionally accepted by Radiology.

Additional recent publications of importance to the field (in chronological order)

1. M Haacke, S. Mittal, Z. Wu, J. Neelavalli, Y.C. Cheng. Susceptibility-weighted imaging: technical aspects and clinical applications, part 1. AJNR **2009** - 30:19-30.
2. Y.C. Cheng, J. Neelavalli, E.M. Haacke. Limitations of calculating field distributions and magnetic susceptibilities in MRI using a Fourier based method. Phys Med Biol. **2009** - 54:1169-1189.
3. S. Mittal, Z. Wu, J. Neelavalli, E.M. Haacke. Susceptibility-Weighted Imaging: Technical Aspects and Clinical Applications, Part 2. AJNR **2009** – 30:232-252.
4. E.M. Haacke, M. Makki, Y. Ge, M. Maheshwari, V. Sehgal, J. Hu, M. Selvan, Z. Wu, Z. Latif, Y. Xuan, O. Khan, J. Garbern. Characterizing Iron Deposition in Multiple Sclerosis Lesions Using Susceptibility Weighted Imaging. JMRI **2009** – 29;537-544. PMCID: PMC2650739.
5. S.R.S. Barnes and E.M. Haacke. Susceptibility Weighted Imaging: Clinical Angiographic Applications. MRI Clinical N Am **2009** - 17;47-61. PMCID: PMC2713115.
6. J. Neelavalli, Y-C.N. Cheng, J. Jiang, E.M. Haacke. Removing Background Phase Variations in Susceptibility Weighted Imaging Using a Fast, Forward-Field Calculation. JMRI **2009** – 29;937-948.

7. Y. Ge, V.M. Zohrabian, E-O. Osa, J. Xu, H. Jaggi, J. Herbert, E.M. Haacke, R.I. Grossman. Diminished visibility of cerebral venous vasculature in multiple sclerosis by susceptibility-weighted imaging at 3.0 T. JMRI **2009** – 29;1190-1194.
8. E.S. Manova, C.A. Habib, A.S. Boikov, M. Ayaz, A. Khan, W.M. Kirsch, D.K. Kido, E.M. Haacke. Characterizing the mesencephalon using susceptibility weighted imaging. AJNR **2009** - 30;569 –574.
9. Q. Yang, J. Liu, S.R.S. Barnes, Z. Wu, K. Li, J. Neelavalli, J. Hu, and E.M. Haacke. Imaging the Vessel Wall in Major Peripheral Arteries using Susceptibility Weighted Imaging: Visualizing Calcifications. JMRI **2009** - 30;357-365. PMCID: PMC2730889.
10. Chavhan, G.B., Babyn, P.S., Thomas, B., Shroff, M.M., Haacke, E.M. Principles, Techniques, and Applications of T2*-based MR Imaging and Its Special Applications1. RadioGraphics **2009**;29;1433-1449.
11. Cheng, Y-C N., Hsieh, C-Y, Neelavalli, J. and Haacke, E.M. Quantifying effective magnetic moments of narrow cylindrical objects in MRI. Phys. Med. Biol. **2009**-54;7025-7044.

C. Research Support.

H133G080064 (Hanks) — 10/01/2008 – 09/30/2010 — 0.00 calendar
National Institute on Disability — $593, 022
and Rehabilitation Research
Neuroanatomical Correlates of Positive Psychology Among People with Traumatic Brain Injury: A Biopsychosocial Model. *A Field Initiated Grant*
Goals: Improve our ability to identify individual characteristics and resources that can be used to facilitate well-being and recovery of function after TBI.

Guerbet (Haacke) — 11/16/2009-08/31/2010 — 0.24 calendar
P904 Stroke Study — $50,000
Goals: To further test a contrast agent on stroke animals to see if it will work sufficiently to indicate areas of angiogensis.

NSF 06-597 (Dong) — 06/01/2008 – 05/31/2011 — 0.60 calendar
National Science Foundation — $270,822
CRI:IAD Acquisition of Research Infrastructure for Knowledge-enhanced, Large-scale Learning of Multimodality Visual Data
Goals: Purchase a major piece of equipment for data storage.

2R01 HL062983-04A2 (Haacke) — 09/01/2008 – 05/31/2011 — 2.40 calendar
National Institutes of Health — $1,560,829
Susceptibility Weighted Imaging (SWI)
Goals: Continue the development of SWI to: a) make it more clinically viable by reducing phase processing artifacts; b) evaluate susceptibility itself by creating a susceptibility map of human tissue; c) study its role as a new MR angiographic method by simultaneously collecting MRA and SWI data; and d) speed up its acquisition time to less than 5 minutes for whole brain coverage, independent of any parallel imaging gain factor.

K08 MH079176A (Behen) — 09/03/2007 – 07/31/2012 — 0.00 calendar
National Institutes of Health /NIMH — $680,483
Structural and Functional Neural Correlates of Early Postnatal Deprivation
Goals: Evaluate the neuroanatomical correlates of early social deprivation (ESD) in human children using both state-of-the-art MRI and PET methods.

Master Research Agreement (Haacke) — 07/01/2009 – 06/30/2012 — 0.60 calendar
Siemens Medical Solutions — $300,000
Research Agreement
Goals: Collect clinical SWI data for trauma, stroke, and vascular disease.

R01 NS041922 (Juhasz) National Institutes of Health/NINDS Longitudinal neuroimaging in Sturge-Weber syndrome"	07/01/2008 – 04/30/2013 $990,000	0.96 calendar

Goals: To study the effects of Sturge-Weber syndrome on the brain over time.

University Of Saskatchewan (Nichol) CIHR Team in Synchrotron Medical Imaging	11/1/2009 – 10/31/2014 $337,300	0.24 calendar

Goals: 1) map iron in fixed human brains to see changes in metal distribution associated with stroke and 2) Compare DCE MRI with SWI to better understand the etiology of vascular damage prior to the appearance of bleeds and quantify changes in elemental distribution associated with vascular permeability.

OVERLAP

No overlap for Dr. Haacke exists.

Ongoing Research Support (RAFOLS)

R01 NS064976-A2 Kreipke (PI) 11/01/09-10/31/14
NIH_NINDS
Role: CO-I
"Molecular Mechanisms of Enhanced Contractility following Traumatic Brain Injury: towards a clinical trial" (Investigates the mechanism by which endothelin receptor antagonists may be useful in the treatment of cognitive deficits following TBI).

VARR&D 1I01RX000224-01 Kreipke (PI) 11/01/09-10/31/12
Role: CO-I
"Poly-trauma following brain injury: towards a combinatorial therapy" (Investigates the effects of multiple pathologies associated with traumatic brain injury on histopathological and behavioral outcome).

VA RR & D Award Rossi/Kreipke (PI) 04/01/08-12/31/11
VA Rehabilitation
Role: CO-I
"Conditioning, microvascular tone & rehabilitation post brain trauma" (Investigates the role of exercise in the control of microcirculation in a rat model of traumatic brain injury).

Ongoing Research Support (Kreipke)

R01 NS064976-A2 Kreipke (PI) 11/01/09-10/31/14
NIH_NINDS
Role: PI
"Molecular Mechanisms of Enhanced Contractility following Traumatic Brain Injury: towards a clinical trial" (Investigates the mechanism by which endothelin receptor antagonists may be useful in the treatment of cognitive deficits following TBI).

VARR&D 1I01RX000224-01 Kreipke (PI) 11/01/09-10/31/12
Role: PI
"Poly-trauma following brain injury: towards a combinatorial therapy" (Investigates the effects of multiple pathologies associated with traumatic brain injury on histopathological and behavioral outcome).

VA RR & D Award Rossi/Kreipke (PI) 04/01/08-12/31/11
VA Rehabilitation
Role: CO-PI
"Conditioning, microvascular tone & rehabilitation post brain trauma" (Investigates the role of exercise in the control of microcirculation in a rat model of traumatic brain injury).

Ongoing (Active) Research Support

NIH/NIDA 5 R01 DA10756 04/10/07-04/09/12
Neurotoxic Amphetamines, Radicals, and 5HT Neurons
The major goal of the study is to determine the mechanisms by which neurotoxic amphetamine-derived reactive oxygen and nitrogen species alter function of dopamine and serotonin neurons through their effects on important phenotypic marker proteins in these neuronal elements.
Role: PI

NIH/NIDA 1 RO1 DA017327 04/01/05 – 03/30/10
Methamphetamine Neurotoxicity and Microglial Activation
The goal of this project is to elucidate the role of microglia in the neurotoxic effects associated with methamphetamine and other neurotoxic amphetamines.
Role: PI

Department of Veterans Affairs Merit Award 03/15/07-03/14/11
Brain Injury by Blast Overpressure: Role of Microglial Activation
The goal of this project is to characterize microglial involvement in brain damage caused by blast overpressure. We have developed a model of blast overpressure, a form of traumatic brain injury, that allows testing of cultured cells and brain slices.
Role: PI

R01 NS064976-A2 Kreipke (PI) 11/01/09-10/31/14
NIH_NINDS
"Molecular Mechanisms of Enhanced Contractility following Traumatic Brain Injury: towards a clinical trial" (Investigates the mechanism by which endothelin receptor antagonists may be useful in the treatment of cognitive deficits following TBI).
Role: Co-I

VARR&D 1I01RX000224-01 Kreipke (PI) 11/01/09-10/31/12
"Poly-trauma following brain injury: towards a combinatorial therapy" (Investigates the effects of multiple pathologies associated with traumatic brain injury on histopathological and behavioral outcome).
Role: Co-I

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix:	Dr.
* First Name:	Jose
Middle Name:	A
* Last Name:	Rafols
Suffix:	PhD

2. Human Subjects

Clinical Trial?	☒ No	☐ Yes
* Agency-Defined Phase III Clinical Trial?	☐ No	☐ Yes

3. Applicant Organization Contact

Person to be contacted on matters involving this application

Prefix:	Ms.
* First Name:	Lisa
Middle Name:	M.
* Last Name:	Ellis
Suffix:	
* Phone Number:	313-577-9120
Fax Number:	313-577-5055
Email:	ak5050@wayne.edu
* Title:	Grant & Contract Officer
* Street1:	5057 Woodward
Street2:	13th Floor
* City:	Detroit
County/Parish:	
* State:	MI: Michigan
Province:	
* Country:	USA: UNITED STATES
* Zip / Postal Code:	48202-4050

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells

* Does the proposed project involve human embryonic stem cells? ☒ No ☐ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://stemcells.nih.gov/research/registry/. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

PHS 398 Modular Budget, Periods 1 and 2

OMB Number: 0925-0001

Budget Period: 1

Start Date: 04/01/2011 End Date: 03/31/2012

A. Direct Costs

	* Funds Requested ($)
* Direct Cost less Consortium F&A	250,000.00
Consortium F&A	
* Total Direct Costs	250,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Modified Total Direct Costs	52	238,600.00	124,072.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): D.H.H.S. Wanda Rayfield 214-767-5249

Indirect Cost Rate Agreement Date: 04/27/2009

Total Indirect Costs: 124,072.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): 374,072.00

Budget Period: 2

Start Date: 04/01/2012 End Date: 03/31/2013

A. Direct Costs

	* Funds Requested ($)
* Direct Cost less Consortium F&A	250,000.00
Consortium F&A	
* Total Direct Costs	250,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Modified Total Direct Costs	52	238,258.00	123,894.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): D.H.H.S. Wanda Rayfield 214-767-5249

Indirect Cost Rate Agreement Date: 04/27/2009

Total Indirect Costs: 123,894.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): 373,894.00

PHS 398 Modular Budget, Periods 3 and 4

Budget Period: 3

Start Date: 04/01/2013 End Date: 03/31/2014

A. Direct Costs

	* Funds Requested ($)
* Direct Cost less Consortium F&A	250,000.00
Consortium F&A	
* Total Direct Costs	250,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Modified Total Direct Costs	52	237,906.00	123,711.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): D.H.H.S.
Wanda Rayfield
214-767-5249

Indirect Cost Rate Agreement Date: 04/27/2009

Total Indirect Costs: 123,711.00

C. Total Direct and Indirect Costs (A + B) Funds Requested ($): 373,711.00

Budget Period: 4

Start Date: 04/01/2014 End Date: 03/31/2015

A. Direct Costs

	* Funds Requested ($)
* Direct Cost less Consortium F&A	250,000.00
Consortium F&A	
* Total Direct Costs	250,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Modified Total Direct Costs	52	237,543.00	123,522.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): D.H.H.S.
Wanda Rayfield
214-767-5249

Indirect Cost Rate Agreement Date: 04/27/2009

Total Indirect Costs: 123,522.00

C. Total Direct and Indirect Costs (A + B) Funds Requested ($): 373,522.00

PHS 398 Modular Budget, Periods 5 and Cumulative

Budget Period: 5

Start Date: 04/01/2015 End Date: 03/31/2016

A. Direct Costs

	* Funds Requested ($)
* Direct Cost less Consortium F&A	250,000.00
Consortium F&A	
* Total Direct Costs	250,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Modified Total Direct Costs	52	237,169.00	123,328.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): D.H.H.S. Wanda Rayfield 214-767-5249

Indirect Cost Rate Agreement Date: 04/27/2009

Total Indirect Costs: 123,328.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): 373,328.00

Cumulative Budget Information

1. Total Costs, Entire Project Period

*Section A, Total Direct Cost less Consortium F&A for Entire Project Period	$	1,250,000.00
Section A, Total Consortium F&A for Entire Project Period	$	
*Section A, Total Direct Costs for Entire Project Period	$	1,250,000.00
*Section B, Total Indirect Costs for Entire Project Period	$	618,527.00
*Section C, Total Direct and Indirect Costs (A+B) for Entire Project Period	$	1,868,527.00

2. Budget Justifications

Personnel Justification	1250-Personnel.pdf	Add Attachment	Delete Attachment	View Attachment
Consortium Justification		Add Attachment	Delete Attachment	View Attachment
Additional Narrative Justification		Add Attachment	Delete Attachment	View Attachment

Personnel

Jose Rafols, Ph.D., Principal Investigator (3.6 cal. mos.) has over thirty years of experience performing studies of brain ischemia and TBI. He will direct and be responsible for facilitating the research design. He will assume responsibility that all experiments are conducted in a proper manner and finalized in a timely fashion. In addition, he will be responsible for the final preparation of manuscripts and grants that directly accompany this work.

Christian Kreipke, Ph.D., CO-Investigator (1.2 cal. mos.) is an emerging investigator in the field of TBI research. He joined Dr. Rafols' laboratory to conduct brain trauma research over three years ago and, hence has finished his post-doctoral training. During the current lab funding, he has enhanced the laboratory's goals by including behavioral analysis and added to the pharmacological experiments designed. Dr. Kreipke has experience in mentoring students, working with the model of brain injury, molecular biology and in assessing animal behavior. He will assist Dr. Rafols in leading the direction of the research.

Mihir Bagchi, Ph.D., CO-Investigator (2.4 cal. mos.) has over thirty years of experience and a wealth of publications in conducting molecular and biochemical experiments. In the past year, as evidenced in our list of manuscripts in the current funding, Dr. Bagchi has already begun working with us. He will oversee and personally conduct many of the Western blots.

Donald Kuhn, Ph.D., (1.2) is a leading expert in behavioral pharmacology and will provide expertise and access to behavioral equipment and in designing pharmacological treatments.

E. Mark Haack, Ph.D., (0.6 cal. mos.) is a leading expert in MRI. He will provide expertise and access to equipment for carrying out all DAI and CBF measurements utilizing MRI.

Christian Reynolds, Graduate Research assistant (12 cal. mos.) will be instrumental in carrying out the behavioral analysis. As evidenced in the proposal, much of our behavioral work is labor intensive and is done 20-30 days consecutively, as well as is conducted during evening hours to correspond with the active cycle of rat's diurnal cycle. Therefore, we feel a dedicated person to this task who will not have to conduct experiments during day hours is needed. Further, one initiative within this proposal is the training of students in neurosciences. This training will be overseen by Dr. Rafols.

OMB Number: 0925-0001

PHS 398 Research Plan

1. Application Type:

From SF 424 (R&R) Cover Page. The response provided on that page, regarding the type of application being submitted, is repeated for your reference, as you attach the appropriate sections of the Research Plan.

*Type of Application:

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

2. Research Plan Attachments:

Please attach applicable sections of the research plan, below.

1. Introduction to Application (for RESUBMISSION or REVISION only)		Add Attachment	Delete Attachment	View Attachment
2. Specific Aims	1240-specific aims.pdf	Add Attachment	Delete Attachment	View Attachment
3. *Research Strategy	1241-research strategy.pdf	Add Attachment	Delete Attachment	View Attachment
4. Inclusion Enrollment Report		Add Attachment	Delete Attachment	View Attachment
5. Progress Report Publication List		Add Attachment	Delete Attachment	View Attachment

Human Subjects Sections

6. Protection of Human Subjects		Add Attachment	Delete Attachment	View Attachment
7. Inclusion of Women and Minorities		Add Attachment	Delete Attachment	View Attachment
8. Targeted/Planned Enrollment Table		Add Attachment	Delete Attachment	View Attachment
9. Inclusion of Children		Add Attachment	Delete Attachment	View Attachment

Other Research Plan Sections

10. Vertebrate Animals	1251-Vertebrate Animals.pdf	Add Attachment	Delete Attachment	View Attachment
11. Select Agent Research		Add Attachment	Delete Attachment	View Attachment
12. Multiple PD/PI Leadership Plan		Add Attachment	Delete Attachment	View Attachment
13. Consortium/Contractual Arrangements		Add Attachment	Delete Attachment	View Attachment
14. Letters of Support		Add Attachment	Delete Attachment	View Attachment
15. Resource Sharing Plan(s)		Add Attachment	Delete Attachment	View Attachment

16. Appendix Add Attachments | Remove Attachments | View Attachments

SPECIFIC AIMS

Traumatic brain injury (TBI) is reportedly the leading cause of death and disability among children and young adults (CDC Report, 2004). TBI is also known as the signature injury in the War on Terrorism. USA Today reported (2007) that 83% of brain injured marines and sailors returning from Iraq suffer cognitive impairments. The combined emotional and financial costs of civilian casualties and those associated with the military as they try to rejoin civilian life is overwhelming. Therefore, there is a compelling need to implement effective therapies to improve the quality of life of those suffering from TBI.

Among multiple sequelae, TBI results in three major pathologies: 1) cerebral edema which leads to a critical rise in intracranial pressure, 2) diffuse axonal injury (DAI) which brings about disruption of neural circuits underlying cognitive and motoric behaviors, and 3) alterations in cerebral blood flow (CBF) that cause a persistent state of hypoperfusion and improper delivery of vital metabolites to neural tissue. While all three pathologies combine to cause substantial morbidity and mortality seen in the clinical setting, how these pathologies influence each other for final outcome is unclear. This laboratory has been successfully funded for the last decade to focus on the role of the endothelin (ET)-1 system in hypoperfusion following TBI. Recently, we have begun studies aimed at testing the effects of endothelin receptor A (ETrA) antagonists, which inhibit vasoconstriction (and hypoperfusion), as possible treatments for improving outcome following TBI. While experimental efficacy data is promising, the mechanism by which ETrA antagonists may exert their influence is not known. In the previous grant submission we continued to test the overall hypothesis that improving blood flow directly after TBI via ETrA antagonism can improve outcome after injury. However, as pointed out by several reviewers, hypoperfusion, alone, likely does not cause the deleterious effects of TBI. Thus, since the first submission we have designed a novel approach that aims to understand the mechanism by which ETrA antagonism may improve both histopathologic and behavioral outcome by focusing on polypathologies (in this case combined hypoperfusion and DAI). The central hypothesis of this grant is that: ***ETrA antagonism reduces the TBI-induced hypoperfusion and diminishes the extent of DAI, the combined effects from this intervention leading to improved histopathologic and behavioral outcomes***. Because the ability of ETrA antagonism to improve CBF has been the source of our previous funding, the present proposal focuses on the effect of hypoperfusion on DAI. Furthermore, since in parallel work in our lab aims to study the efficacy of ETrA antagonists in ameliorating behavioral deficits following TBI, this proposal is designed to augment his work with a mechanistic rationale for pursuing clinical trial with ETrA antagonists. We will test our hypotheses through the following Specific AIMS:

SPECIFIC AIM 1 tests the hypothesis that the observed decrease in CBF following TBI, alone, neither causes histopathologic changes nor behavioral deficits.

SPECIFIC AIM 2 tests the hypothesis that, in the presence of TBI, ET-1 signaling through ETrA contributes to the sequelae leading to DAI.

SPECIFIC AIM 3 tests the hypothesis that Clazosentan, a clinically relevant ETrA antagonist, diminishes the extent of TBI-induced DAI, thus improving both histolopathologic and behavioral outcomes following injury.

In order to accomplish these Specific AIMs we have recruited an interdisciplinary team of experts which, together, will provide new data on the mechanism by which ETrA is directly involved in the overall pathotrajectory of TBI. This data will be used in conjunction with already funded pre-clinical efficacy data using ETrA antagonists to provide further rationale for the use of this class of drugs in the clinics. In addition this data will be used to guide our developing clinical application of ETrA antagonists after TBI, thus providing a rapid "bench to bedside" translation of our work to help those suffering the effects of brain injury.

RESEARCH STRATEGY: BACKGROUND AND SIGNIFICANCE

Traumatic Brain Injury (TBI) and Brain Pathology: Why focus on endothelin pharmacology?

TBI results in several major histopathologic events, including among others: cerebral edema which leads to a critical rise in intracranial pressure, DAI which brings about disruption of neural circuits underlying cognitive and motoric behaviors, and alterations in the brain's microcirculation that cause a persistent state of hypoperfusion and improper delivery of vital metabolites to neural tissue which in turn exacerbates neuronal injury leading to secondary injuries. In closed head TBI incidents in humans, all these events are thought to accrue and contribute to the ensuing morbidity and mortality encountered in clinical settings. The experiments designed in the present proposal expand upon our previously funded work using ET-1 receptor A (ETrA) antagonists by exploring novel mechanisms by which ETrA antagonism may cross pathological boundaries, producing not only reductions in hypoperfusion, but also in DAI. While ET-1 receptor antagonism is widely used therapeutically in clinical trials involving kidney, lung and heart vasospasm (Benigni and Remuzzi, 2009)), a similar approach to improve cerebral vasospasm after stroke or trauma remains to be implemented. Using a well validated, closed head acceleration impact rodent model, our laboratory has demonstrated a key role of ET-1 and its receptors in causing a state of chronic hypoperfusion after TBI (Rafols et al, 2007). In the present proposal we address the mechanistic underpinnings of the ET-1 receptors in the non-injured and injured brain and provide insights into novel pharmacological therapies that may be readily translated from laboratory bench into the clinical setting.

Endothelin (ET) - 1

Our laboratory and others have established that alterations in ET-1 metabolism after TBI are causally related to the sustained hypoperfusion affecting the brain microcirculation (Armstead, 1996; Kasemari & Armstead, 1997; Kasemari & Armstead, 1997; Armstead, 1999; Armstead, 2001; Rafols et al., 2007). Endothelins (ETs) are peptides which at high concentrations exert an extremely potent and long-lasting vasoconstriction (ET-1 vasoconstrictive effects are X10 greater than those of angiotensin-1, Warner et al., 1994b). The genes that encode for ETs are present in human, porcine, murine and rat cells (Inoiue et al., 1989; Saida et al., 1989). ETs are expressed in a variety of tissues: neurons, adrenal gland, lung, heart and kidney (Masaki, 1993), as well as endothelial cells of porcine aorta from which they were first isolated (Yanagisawa et al., 1988). Synthesis of ETs has been shown in astrocytes and macrophages (Hori et al., 2001; Petrov et al., 2002a). However, *while the function of the ETs and their receptors in the microvascularization are well known, their effects in non-vascular tissue (e.g., neurons, glia) remain unclear*.

There are three isoforms of the peptide, ET-1, ET-2 and ET-3, each containing 21 amino acids, 2 disulfide bridges and 1 hydrophobic C-terminal end with a tryptophan in position 21 (Saida et al., 1989; Yanagisawa and Masaki, 1989). Despite their structural similarity, ET-1 and ET-2 are almost equally potent, while ET-3 is less potent (Zimmermann and Seifert, 1998). The ETs also have different affinities to their receptors, i.e., the affinity of ETrA to ET-1 and ET-2 is two orders of magnitude higher than that to ET-3; ETrB, on the other hand, shows similar affinity to all three isoforms (Sakurai et al., 1992). Furthermore, when ETs bind to their receptors they dissociate very slowly indicating a long lasting effect (Hirata et al., 1988; Marsault et al, 1993). We have shown that there is a characteristic shift in ETs receptor cellular localization in brain after trauma (Kallakuri et al., 2007a and b), implying these receptors in mechanisms may relate not only to microvascular responses but also, as suggested by others (Zimmermann and Seifert, 1998), to the development of secondary brain injury.

Endothelin Receptors

The endothelin receptors belong to the superfamily of G-protein-coupled heptahelical receptors and activate phospholipase C (PLC) (Arai et al., 1990; Sakurai et al., 1990). They have a molecular weight of approximately 47 kDa and contain seven transmembrane domains of 20-27 hydrophobic amino acid residues (Warner et al, 1994b). In general, ETs contribute to the development of chronic vasospasm via activation of both receptors (Shigeno et al, 1995) (detailed below).

ETrA. This receptor has been detected in vascular smooth muscle (SM) in brain blood vessels (Hori et al., 1992), neurons (Kurokawa et al., 1997; Kallakuri et al, 2007a; 2010), astrocytes and endothelial cells (Nakagomi et al., 2000). When ETs are released they bind to ETrA on SM. At the time of ET-1 release from the endothelium, an intermediate form of the peptide, called big-ET, is also released. Big-ET is converted to ET-1 by endothelin-converting enzymes (ECEs) on the surface of SM (D'Orleans-Juste et al., 1990; McMahon et al., 1991), and this ET-1 binds also to ETrA in SM. The resulting SM contraction is Ca^{2+}-dependent and may

be mediated in part by activation of PLC (Povlishock et al., 1983) and phosphorylation of contractile proteins in SM such as calponin (Kreipke et al., 2006;2007,a,c; 2009).

Alterations in the expression of ETrA were observed in several brain pathological conditions. mRNA for ETrA was significantly upregulated 3 and 7 days following subarachnoid hemorrhage (Itoh et al., 1993) or after kainic acid-induced brain damage (Sakurai-Yamashita et al., 1997). We have published that ETrA mRNA as well as protein are upregulated during the hypoperfusion phase brought about by TBI (Kreipke and Petrov 2005; Kallakuri et al., 2007a, b; Kreipke et al., in press).

Effects of ETrA blockade. BQ-123 (cyclo-D-α-aspartyl-L-prolyl-D-valyl-L-leucyl-D-tryptophyl) has been shown to have a high affinity for ETrA and a potent action in attenuating the contractile function of ETrA (Ihara et al., 1992; Ishikawa et al., 1992). It has a high affinity for ETrA and, even at higher doses, does not affect body temperature, body weight, mean blood pressure or heart rate (Hirose et al., 1995). In addition it has a prolonged effect (days) after a single ICV injection (Hirose et al., 1995; Josko et al., 2001; Yip and Krukoff, 2002). For these reasons BQ-123 has been suggested as a potential therapeutic agent for improving blood flow after brain trauma.

Hypertension associated with increased contractility of blood vessels in spontaneously hypertensive rats was attenuated by systemic application of BQ-123 (Morel and Godfraind, 1994). ET-1-induced hypertension was also attenuated significantly following blockade with this antagonist (Warner et al, 1994a). In addition, the increase in arterial pressure observed following restraint stress was reduced by ICV application of BQ-123 (Yip and Krukoff, 2002). In the heart following ischemia/hypoperfusion, BQ-123 effectively antagonized the coronary constrictive effect of ET-1 and improved functional recovery during reperfusion (Han et al., 1995). Application of this antagonist was also protective in ischemic acute renal failure in rats (Mino et al., 1992).

Attenuation of ETrA in brain resulted generally in an increase in the cross-sectional diameter of cerebral arteries, especially following subarachnoid hemorrhage (Ishikawa et al, 1994; Warner et al, 1994b). In vivo blockade of ETrA with BQ-123 ameliorated the outcome of autoimmune encephalomyelitis, possibly by reducing the hypoperfusion observed in this disease (Shin et al., 2001). Intracisternal application of BQ-123 abolished the reduced cerebral blood flow (CBF) induced by subarachnoid hemorrhage (Clozel and Watanabe, 1993). Blockade of ETrA with this antagonist also led to normalization of neurological performance and CBF within two day post cardiac arrest (Krep et al., 2000). In addition, in combination with acetylcholine, application of BQ-123 induced a widespread significant increase (in some cases up to 50%) in CBF (Granstam et al., 1998). Blockade of ETrA resulted in reduction of the lesion due to attenuated vasoconstriction following cold injury to the brain (Gorlach et al., 2001) or focal stroke (Barone et al, 2000).

In the current funding period we sought to test the effects of ETrA blockade on microvascular tone following TBI. We previously showed that an ICV injection of 40μg BQ-123 prior to TBI was able to ameliorate the TBI-induced hypoperfusion (Kreipke et al., 2010). We also showed that ETrA antagonism reduced the extent of cell injury (Kreipke et al., 2010) and improved behavior (Reynolds et al., 2001).

ETrB. Of the two receptors, ETrB is the more abundant in brain tissue (Hama et al., 1997). It has been detected in astrocytes (MacCumber et al., 1990; Kallakuri et al., 2007), neurons (Nakagomi et al., 2000), microglia (Sakurai-Yamashita et al., 1997) endothelial cells and vascular SM (Peters et al., 2001; Kallakuri et al., 2007). Activation of ETrB triggers events similar to those described during activation of ETrA. More specifically, activation of ETrB causes increase in cytosolic Ca^{2+}, stimulation of extracellularly-regulated kinase pathways (Marsault et al., 1990; Lazarini et al., 1996) and modulation of cytoskeletal actin organization (Cazaubon et al., 1997; Koyama and Baba, 1996). ETrB is distributed to selectively vulnerable areas of the CNS such as the hippocampus and cerebral cortex (Bousso-Mittler et al., 1989; Kloog and Sokolovsky, 1989; Williams et al., 1991; Kallakuri et al, 2007). Moreover, distribution receptor binding studies after subarachnoid hemorrhage have shown a shift of receptor distribution from ETrA to ETrB (Roux et al., 1995). We have shown that TBI results in an upregulation of ETrB 24 hours following injury, this upregulation occurring after the initial increase in ETrA (Kreipke et al., in press).

The effect of ETrB activation on microvascular tone is somewhat controversial. It has been reported to act as both a vasodilator (Randall et al., 1989; Hasunuma et al., 1990; Fukuroda et al., 1994; Ivy et al., 1994; Sato et al., 1995) and a vasoconstrictor (Clozel et al., 1992; Harrison et al., 1992; Moreland et al., 1992; Teerlink et al., 1994). While it has been suggested that, due to this disparity, two subtypes, ETrB1 and ETrB2 of ETrB exist, only subtypes ETrA and B have been cloned (Cazaboun and Courand, 1998; Nakagomi et al., 2000; Ho et al., 2001). In endothelium, ETrB is thought to mediate vasodilation through ET-1 clearing and nitric oxide (NO) release (reviewed in Pollack and Schneider, 2006). The activity of ETrB may be related to its localization.

In endothelium, ETrB is thought to mediate vasodilation through ET-1 clearing and nitric oxide (NO) release (reviewed in Pollack and Schneider, 2006). However, in one study it was shown that ETrB exerts a vasoconstrictive force within pulmonary SM (Perreault and Baribeau, 1995). Another study showed that an initial dose of 1µmol of BQ-3020 applied directly to pial arterioles resulted in vasodilation while subsequent doses in the same vessel given 10 min apart resulted in no effect followed by vasoconstriction (Touzani et al., 1997).

Effects of ETrB blockade. ETrB antagonism, like ETrB function, is somewhat controversial. BQ-788 has been shown to attenuate increased perfusion resistance in tumor (HSN fibroblastoma) vasculature (Bell et al., 1999). In another study, elevation of blood pressure induced by ET-1 application was eliminated by BQ-788 (Ishikawa et al., 1994). Several other studies, however, have shown that ETrB antagonism has no effect on blood pressure (reviewed in Pollack and Schneider, 2006). One study showed that, in pial vessels, BQ-788 abolished BQ-3020 (a selective ETrB agonist)-induced vasodilation (Touzani et al., 1997).

ETrA/B in neurons. While ET-1 and ETrA vasoconstrictive functions have been elucidated (Closel et al 1992, Teerlink et al 1994), the potential role of the receptors in neurons in different pathological states remains unknown. Several studies have supported a role for ETrA in maintaining both neuronal cell body and axonal integrity. Dos Santos et al (2007) showed that ET-1 injection produced a dose-dependent increase in axonal damage. One report suggested that ET-1 antagonism decreased DAI in spinal cord injury (Uesugi et al., 1998). Sato et al (1998) showed that administration of ETrA antagonists resulted in fewer HSP70 labeled cortical neurons after acute cortical neuronal injury. Administration of the ETrA antagonist BQ123 before or after ischemia increased hippocampal CA1 neuronal survival in gerbils subjected to transient global ischemia (Feuerstein et al 1994). Taken together, there is compelling evidence that ET-1, acting through ETrA may exert a direct effect on neuronal cell body and axonal integrity and guidance which would provide strong evidence as to why ETrA antagonists may be effective in improving outcome following TBI. However, to date, no one has conducted a comprehensive study as to the effect of ETrA antagonism on DAI and cell body integrity following TBI.

While the above reports indicate a role for ETrA in mediating cell/axonal injury, several reports support a role for ETrB in neuronal survival. Ehrenreich et al (2000) detected increased apoptosis in neuronal cultures from hippocampus of ETrB deficient rats and increased apoptosis in hippocampal dentate gyrus in association with loss of neuronal ETrB immunoreactivity. Siren et al (2002) found large cortical infarcts and hippocampal apoptosis in ETrB deficient rats subjected to hypoxia-ischemia. While taken together these works support a role for ETrA and ETrB in mechanisms of nerve cell injury/survival, it is presently unclear whether the substantial upregulation in neurons after TBI reported here may underlie such mechanisms.

Rationale for the Investigation. Previous *studies on ETrA modulation to reduce cell injury post TBI are few due to the fact that translatable outcome measures such as Diffuse Tensor Imaging (DTI) MRI to detect extent of DAI both experimentally (i.e, rodent TBI models) and in the clinics had not been implemented. In addition, with exception to our recent publication (Kreipke et al., 2010), to date no one has tested the effects of ETrB antagonism on outcome after TBI. Thus our proposal is designed to close these gaps in our knowledge.*

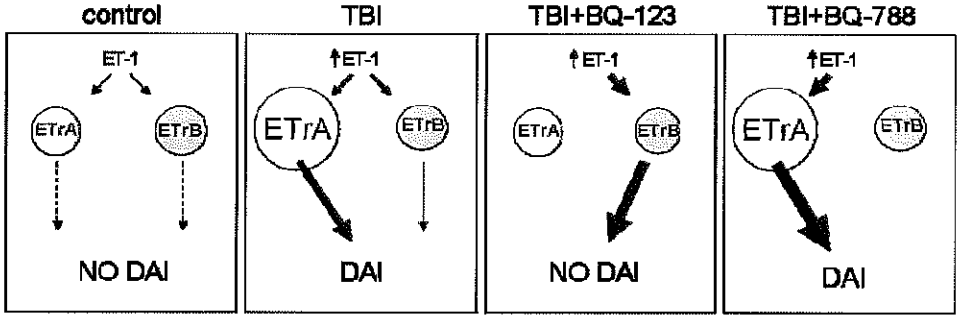

Figure 1. Schematic summarizing the central hypothesis that ETrA antagonism decreases DAI. In control, ET-1 signals through both ETrA and ETrB (both receptors present at low levels in neurons) and axons remain intact. By four hrs post TBI, we have demonstrated a significant upregulation of ET-1 and ETrA in cortical and hippocampal neurons (Kallakuri et al., 2010). Does this upregulation lead to enhancement of cell injury mechanisms leading to DAI? To address this issue we conducted parallel studies and demonstrated DAI in the same centers and in corpus callosum at the same post TBI time (Rafols et al, 2007). In addition if ETrA is blocked using BQ-123, ET-1 signaling shifts to ETrB which may enhance the ability of ETrB to protect neurons, thus decreasing DAI (preliminary results). Conversely, blocking ETrB with BQ-788 shifts ET-1 signaling towards ETrA, thus exacerbating cell injury and DAI.

ET receptor antagonism and clinical therapeutics. Beginning in the early 1990s, endothelin was studied in humans for its potential role in the clinical setting (Vierhapper et al., 1990; Baldys-Waligorska and Szybinski, 1992). Since then, endothelin has been a target for studying a host a pathological states that include disruption of blood flow, including hypertension (Baldys-Waligorska and Szybinski, 1993), hepatorenal syndrome (reviewed in Epstein, 1994), heart failure (Sakai et al., 1996) and decreased cerebral blood flow and hypoxia (Therkelsen et al., 1994). In 1995 Luscher and Wenzel published one of the first reviews which characterized ET-antagonists as potential clinical therapeutics for vascular disorders (Luscher and Wenzel, 1995). In 1999, Benigni and Remuzzi published a follow-up which summarized data from pre-clinical and clinical studies which showed promise for specific ETrA antagonists in controlling hypertension. Bosentan, a mixed antagonist (ETrA and B) was discussed and clinical trial suggested that the potential opposing effects of ETrA and B may render Bosentan less effective (Benigni and Remuzzi, 1999). In 2003, it was reported that, after thorough investigation of ongoing clinical trial, Bosentan had some success in control of pulmonary arterial hypertension, however was not more effective than other, non-endothelial specific drugs (Krum and Liew, 2003). Once again, this may be attributed to Bosentan being a mixed antagonist. At the 2007 10th international symposium on endothelin (ET-10) in Bergamo, Italy, several investigators pointed out that while mixed antagonists have had some effects in pre-clinical studies, overall these agents have had little to no effect in the clinical setting. Therefore, it was proposed that specific ETrA antagonists may be more useful.

The first report on a new drug, produced by Actelion Pharmaceuticals, INC in Switzerland, Ro 61-1790 [5-methyl-pyridine-2-sulfonic acid 6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)-2-(2-1H-tetrazol-5-yl-+ ++pyri din-4-yl)-pyrimidin-4-ylamide] was published in 1997 (Roux et al., 1997). It was found to be 1000-fold more selective for ETrA than ETrB. It was suggested that Ro 61-1790, which was renamed Clazosentan, may be useful for TBI (Sato and Noble, 1998), ischemia (Dawson et al., 1999), and subarachnoid hemorrhage (Gorlach et al., 2001). In 2006, clazosentan was included in a clinical trial to prevent vasospasm following hemorrhage (Uhlmann, 2006). Interestingly, this drug has been shown to have little effect in non-brain areas (Vuurmans et al., 2004). Therefore, selective ETrA antagonism provides a great potential for therapeutic intervention following TBI. Once again, however, the mechanism of action is not understood.

PRELIMINARY STUDIES TO THE PROPOSED AIMS IN THE CURRENT PROPOSAL

SPECIFIC AIM 1 tests the hypothesis that the observed decrease in CBF following TBI, alone, neither causes histopathologic changes nor behavioral deficits.

This AIM assesses whether a 40% reduction of CBF following TBI alone is sufficient to cause cell injury or whether TBI is a prerequisite for decreased CBF-induced cell injury. It has been previously suggested that in the presence of DAI and other pathologies associated with TBI, secondary cell injury may occur in the state of "mild" ischemia (reviewed in Siesjo, 1993). Therefore we first tested whether a 40% reduction of CBF without TBI could cause cell injury.

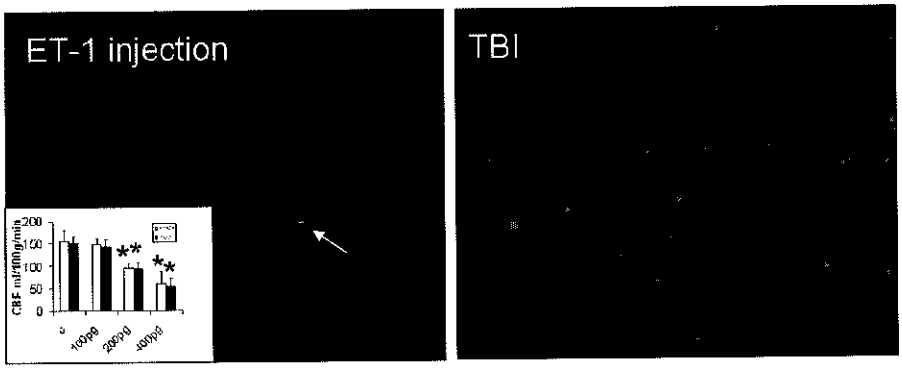

Figure 2. Effect of ~40% reduction of CBF on neuronal integrity both alone and in the presence of TBI. We first determined the appropriate dose of ET-1 that would cause an ~40% reduction in CBF in the non-injured brain. To determine the extent of cell injury, 200pg ET-1 was injected iv and 4 hours later, coronal sections through sensorimotor cortex (smCx) were obtained and stained histochemically with FluoroJade (FJ), a marker of cell membrane integrity. TBI brains were dissected at 4 hours post TBI and stained identically. Results show that while ET-1 injection caused negligible FJ staining in smCx, significant FJ staining of cell bodies was found in superficial layers II-III of smCx after TBI. This suggests that while a 40% reduction in CBF alone is not sufficient to cause cell injury, TBI-induced hypoperfusion causes significant cell injury.

SPECIFIC AIM 2 tests the hypothesis that, in the presence of TBI, ET-1 signaling through ETrA contributes to the sequelae leading to DAI.

In order to test proof of concept and feasibility, we have already begun this aim. The following preliminary data show results from a limited set of animals supporting the notion that BQ-123 decreases DAI.

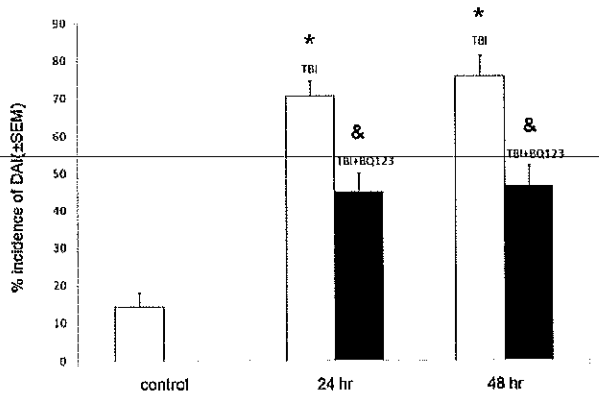

Figure 3. Incidence of DAI (assessed by βAPP immunostaining and morphometric methods) in corpus callosum was significantly reduced in BQ-123-treated injured rats, compared to non treated TBI animals. In addition BQ-123 treatment alone did not induce DAI. Thus, ETrA antagonism significantly improved both TBI-induced vasospasm and DAI (*=p<0.05 compared to control; &=p<0.05 as compared to TBI)

A novel aspect of this revised application is incorporation of DTI-MRI to assess DAI. The distinct advantages of this approach include: 1) DAI progression can be monitored over time in the same animals, and 2) DTI is currently being used in our facility in humans and therefore presents a unique opportunity for translational research. Therefore, we have also included feasibility data in assessing DAI in our animals.

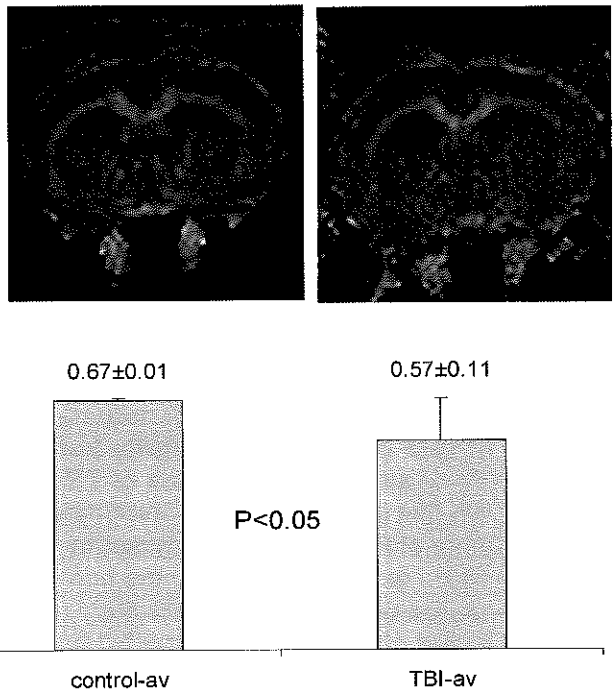

Figure 4. DTI was used to detect DAI in animals 4 hours post TBI. The 2D spin echo DTI with EPI sequence was used *in vivo* for brains 4 h post injury versus control with 6 gradient directions, TR= 850 ms, TE=58 ms, b=0 and 800 sec/mm^2, diffusion gradient duration/separation=5/20 ms, FOV=32x32 mm^2, matrix size=128x128, 13 slices in 1mm, Nacq=1, Nrepeat=8, and total imaging time 6m4s. Effective band width=200 kHz. Images are representative of control (sham operated) and TBI animals. Quantitative data show the average of 6 measurements of frequency of axonal tracts (FA) +/- SEM in optic tract per animal (n=4 per group). Results indicate that TBI results in an approximately 15% loss of axonal fiber tracts. In this proposal we will extend this analysis to include measurements in corpus callosum as well as smCx and dorsal hippocampus (Hipp), centers known to be important for cognition. Similarly we will also assess DAI in animals treated with ETr antagonists v. vehicle as control.

SPECIFIC AIM 3 tests the hypothesis that Clazosentan, a clinically relevant ETrA antagonist, diminishes the extent of DAI following TBI, thus improving both histolopathologic and behavioral outcomes following injury.

While we have not tested the effects of Clazosentan on DAI as of yet, we have, in a limited number of animals, tested its effects on other histopathologic outcomes. Included, here, is data showing that Clazosentan can significantly reduce cell injury as determined by FluoroJade labeling.

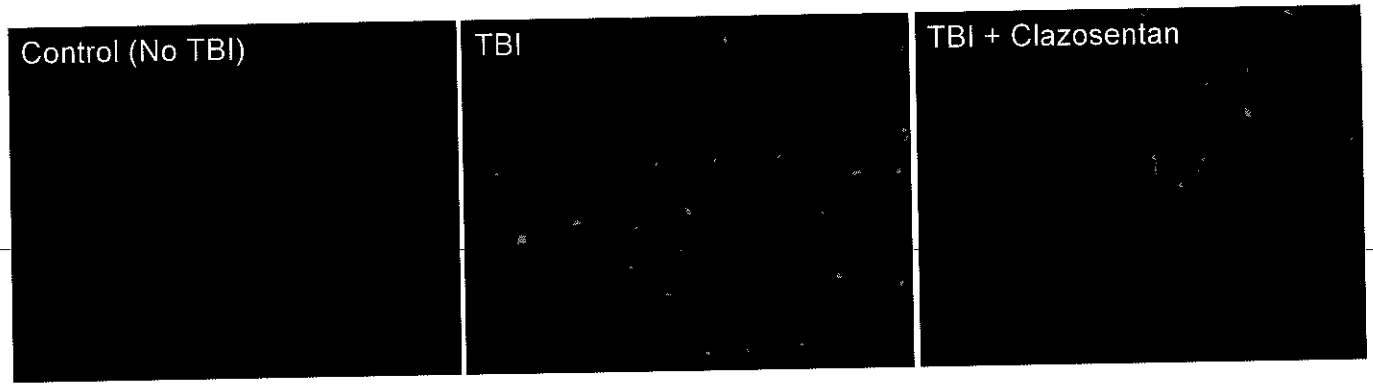

Figure 6. Effect of Clazosentan on cell injury following TBI. In order to determine the effect of blocking hypoperfusion on TBI-induced cell injury, we injected 1.0 mg/kg Clazosentan 30 min after TBI and measured the extent of cell injury using FJ staining. Compared to TBI, Clazosentan caused a significant reduction in FJ cell labeling in layers II-III of smCx, suggesting that blocking ETrA can improve (albeit not entirely) the amount of cell damage that occurs following TBI. Residual neuronal injury detected in Clazosentan treated animals is likely due to initial biomechanical insult. Both TBI and TBI+Clazosentan groups were analyzed at 24 hours post TBI.

RESEARCH DESIGN AND METHODS

SPECIFIC AIM 1 tests the hypothesis that the observed decrease in CBF following TBI, alone, neither causes histopathologic changes nor behavioral deficits.

Rationale

While research in our laboratory has been driven by the assumption that hypoperfusion is contributing to poor outcome following TBI, its precise role in other sequelae is poorly understood. We have observed an approximately 40% reduction in CBF (both by LDF and ASL-MRI methodology) sustained up to 48 hrs post injury (Rafols et al., 2007). However, we have not yet determined whether this, alone, is sufficient to cause the histopathologic and behavioral changes seen after TBI. Therefore the following experiments were designed to help elucidate the influence of reduced CBF on outcome:

<u>Experiment 1.1:</u> Determine the effect of a 40% reduction in CBF in the absence of TBI on neuronal integrity.

In normal (No TBI) animals, we will first determine the dose of intracerebroventricular (ICV) infusion of ET-1 over time which causes an approximately 40% reduction in CBF and that is maintained up to 48 hours (the duration of hypoperfusion seen after TBI). CBF will be measured by ASL-MRI as in AIM1. Once this dose is determined, we will inject the drug, wait 4 hours and collect tissue as described in General Methods. Analyzes for FJ staining, HSP-70, TUNEL, and caspase-3 immunoreactivity, all markers of cell injury/death will be carried out. Data from this experiment will be compared with those from animals receiving saline injection.

Expected Results: Based on our preliminary results, we predict that a 40% reduction of CBF in the absence of TBI will not be sufficient to cause a significant amount of cell injury.

Possible pitfalls, alternative approaches: As previously stated, ET-1 can elicit direct effects on neurons and, hence, ET-1 injections may cause changes in neuronal integrity independent of CBF changes. However, it should be pointed out that the consensus of literature suggests that ET-1 participates in both cell death and survival mechanisms and, therefore, any direct effects may not be detected. Further, our provided preliminary data does not show appreciable cell injury with ET-1 injections, alone. In any case, we would be prepared to select another vasoconstrictor, such as vasopressin, and repeat the experiment to confirm that any changes that may be detected are likely due to CBF changes and not to ET-1 elicited changes in neurons.

Experiment 1.2: Determine the effect of a 40% reduction in CBF in the absence of TBI on behavioral outcome.
We will inject ET-1, ICV, at a dose as determined above that elicits a 40% reduction in CBF up to 48 hours following TBI. We will then test the animals for 21 days on a radial arm maze to assess cognitive function. This data will be compared with that taken from animals receiving saline injections to determine whether a decrease in CBF, alone, is sufficient to cause behavioral deficits.
Expected Results: Based on our preliminary results (Experiment 1.1), we predict that a 40% reduction of CBF in the absence of TBI will not be sufficient to cause a significant amount of behavioral deficits.
Possible pitfalls, alternative approaches: As above.

SPECIFIC AIM 2 tests the hypothesis that, in the presence of TBI, ET-1 signaling through ETrA contributes to the sequelae leading to DAI.

Rationale
Most ischemia investigators would argue that ~40% reduction of blood flow is not sufficient to cause cell injury. In fact, our own preliminary data presented here, in AIM 1 reaffirms this. However, enhanced ET-1 signaling directly following primary biomechanical insult may contribute to DAI.

Experiment 2.1: Determine the effect of ET-1 ICV injection on DAI.
In order to test whether ET-1, alone, elicits DAI, here we will inject ET-1 ICV as in AIM 1 experiments. Vehicle injection animals will serve as controls. No animal will receive TBI. 48 hours following injection we will sacrifice animals and harvest brains for sectioning. This time point is chosen due to published data from our laboratory and others showing that extensive DAI is produced by 48 hours post TBI (reviewed in Rafols, 2007 and Povlishock et al., 2007). DAI will be quantified as described in General Methods. Data will be compared across groups and with already published data using TBI animals to determine whether ET-1 signaling is sufficient to cause DAI.
Expected Results: Based on our preliminary data and the known association between biomechanical forces elicited by TBI and DAI, we do not predict that ET-1 signaling, alone, contributes to DAI.
Possible pitfalls, alternative approaches: In the past we have used β-APP staining as a method to assess DAI, a technique widely used in the field of axonal injury. However, some argue that stain variability and sampling error contributes to low reliability in using this method. Therefore as an adjuvant measure we have recruited Dr. M. Haacke, a world-renowned expert in MRI to assist in using Diffuse Tensor Imaging (DTI) which is becoming an industry standard for assessing DAI. Furthermore, this technique has direct application into the clinic as it is an approved method for assessing DAI in humans.

NOTE: The following set of experiments is designed to determine a causal relationship between ET-1 signaling and DAI.

Experiments 2.2a-e: Determine whether ET-1 signaling through its receptors, A and/or B, contributes to DAI.

Experiment 2.2a: Determine the effect of ET-1 ICV injection on DAI following TBI.
Here we will repeat experiment 2.1, however we will inject ET-1 30 minutes prior to inducing TBI. Saline vehicle will be injected for control. This experiment is designed to test whether enhanced ET-1 signaling can exacerbate TBI-induced DAI.
Expected Results: If in fact, activation of the ET-1 system influences the extent of DAI, we then predict that ET-1 injection following TBI might exacerbate the TBI-induced DAI. Based on preliminary data, we anticipate that ET-1 injection does increase the extent of DAI.
Possible pitfalls, alternative approaches: ET-1 injection will, presumably, have affinity to both ETrA and B. In addition as referenced above, ETrA has been associated with cell injury and ETrB has been associated with cell survival, thus the results of this experiment may be inconclusive. However the following 4 experiments (**2.2b-e**) are designed to tease out whether ETrA or B may contribute to DAI.

Experiment 2.2b: Determine the effect of ETrA antagonism on DAI following TBI.
In order to block ET-1 signaling through ETrA BQ-123, a selective, peptidergic ETrA antagonist will be injected IV 30 minutes prior to TBI. Vehicle injection will be used for control. In order to test a more clinically applicable

situation we will, in another set of animals, inject BQ-123 at 30 minutes, 2 hours, 12 hours, and 24 hours post TBI. In all cases, three different doses (0.1, 1.0 and 10 mg/kg) of BQ-123 will be used, these doses being based on previously published data from our laboratory (Kreipke et al., 2010). DAI will be assessed using both β-APP staining and DTI (see General Methods).

Expected Results: Based on our preliminary data, we predict that IV injection of BQ-123 will reduce the extent of DAI. Further, we predict that, given that DAI appears within 1 hour post TBI and progressively increases for up to 48 hours, successive injections (e.g., 30 minutes, 2 hours, 12 hours, 24 hours) will result in a lessening of effect (i.e., the 30 minute injection will diminish DAI more effectively than the 24 hours injection).

Possible pitfalls, alternative approaches: It may be difficult to determine a significant difference between each group (e.g., the 30 minute injection post TBI may not yield significantly different results from those of the 12 hour). However, we do not anticipate this being a problem since an overall trend will contribute to an understanding of how ET-1 signaling through ETrA may influence DAI. Another caveat to this experiment is that it will still be difficult to interpret whether ETrA antagonist-induced blockade of ET-1 signaling is directly influencing DAI or whether it is due to improvement in blood flow which could lead to a sparing of axonal integrity through reduced hypoxia and increased metabolite delivery. However, the goal of this experiment is to determine whether ET-1 signaling participates in the sequelae leading to DAI and therefore this caveat should not diminish the overall ability to resolve the hypothesis.

Experiment 2.2c: Determine the effect of ETrA antagonism on ET-1 injection followed by TBI-induced DAI.

We will conduct this experiment as in 2.2b, however ET-1 ICV injection will be given 30 minutes following ETrA antagonist delivery. Saline injection in lieu of ET-1 will be used as control.

Expected Results: By blocking ET-1's ability to bind ETrA thus enhancing ETrB signaling, we predict that DAI will be diminished.

Possible pitfalls, alternative approaches: We acknowledge that dosing will be critical in order to effectively overcome binding of the ligand to ETrA. However, we are prepared to include a dose response of BQ-123 in order to achieve this. In this manner, we may be able to detect increasing efficacy of drug.

Experiment 2.2d: Determine the effect of ETrB antagonism on DAI following TBI.

We will conduct this experiment as in 2.2b, however BQ-788, a peptidergic, selective ETrB antagonist will be used instead of BQ-123. Data will be compared to already published data in TBI only animals to determine the effect of ETrB antagonism on DAI after injury.

Expected Results: Based on our preliminary data, we predict that IV injection of BQ-788 will exacerbate DAI. Further, we predict that, given that DAI appears within 1 hour post TBI and ensues for up to 48 hours that successive injections (e.g., 30 minutes, 2 hours, 12 hours, 24 hours) will result in a lessening of effect (i.e., the 30 minute injection will increase DAI more effectively than the 24 hours injection).

Possible pitfalls, alternative approaches: same as in experiment 2.2b.

Experiment 2.2e: Determine the effect of ETrB antagonism on ET-1 injection followed by TBI-induced DAI.

We will conduct this experiment as in 2.2d, however ET-1 ICV injection will be given 30 minutes following ETrB antagonist delivery. Saline injection in lieu of ET-1 will be used as control.

Expected Results: By blocking the ability of ET-1 to bind to ETrB, thus enhancing ETrA signaling, we predict that we will exacerbate DAI.

Possible pitfalls, alternative approaches: As in experiment 2.2c, we are prepared to conduct a dose response for BQ-788 to overcome the effect of ET-1 binding.

SPECIFIC AIM 3 tests the hypothesis that Clazosentan, a clinically relevant ETrA antagonist, diminishes the extent of DAI following TBI, thus improving both histolopathologic and behavioral outcomes following injury.

Rationale

Ultimately, the long range goal of our laboratory is to not only determine the basic science mechanisms by which ETrA antagonists may be useful in the clinic for head trauma patients, but also to discover a clinically relevant treatment that can be readily used. Therefore, aided by data from the previous two AIMs using BQ-123, in this AIM we will implement Clazosentan, a highly specific ETrA antagonist which is currently undergoing clinical trial for vasospasm following subarachnoid hemorrhage (Macdonald, 2008). The following

experiments are designed to recapitulate findings from AIMs 1 and 2 and to extend those findings to determine whether Clazosentan shows promise for moving forward towards clinical trial.

Experiment 3.1: Determine the effect of Clazosentan on DAI following TBI.
Clazosentan will be injected IV 30 minutes post TBI using the most effective dose and time point seen in AIM 2. Vehicle injection and sham operation will be used for controls. DAI will be assessed using both β-APP staining and DTI (see General Methods). Data will be compared with vehicle injected and non-TBI animals.
Expected Results: Based on our preliminary data using BQ-123, we predict that Clazosentan will reduce the extent of DAI.
Possible pitfalls, alternative approaches: While we aim to utilize data gained in AIM 2 to guide this experiment, ultimately we may need to adjust the dose of Clazosentan. However, as we have extensive experience in dose response experiments, we do not predict this to be a major caveat.

Experiment 3.2: Determine the effect of Clazosentan on histopathologic outcome following TBI.
We will inject Clazosentan (effective dose determined in AIM 2) 30min after TBI. Brain tissue will be collected and processed for multiple histologic outcomes as described in General Methods. This data will be compared with vehicle injected and sham operated animals.
Expected Results: Based on preliminary results, we predict that Clazosentan will decrease the extent of histopathology seen following TBI.
Possible pitfalls, alternative approaches: Once again, we may need to adjust the dose of Clazosentan. Furthermore, given that we are measuring multiple indices of cell injury that span several mechanisms (e.g., apoptosis, cell membrane injury, etc) it may be necessary to develop an overall score for cell injury that encompasses all techniques. In such a case, we will work closely with Wayne State University's biostatistical core facility to most accurately determine appropriate statistical analysis.

Experiment 3.3: Determine the effect of Clazosentan on behavioral outcome following TBI.
Clazosentan will be injected IV 30 minutes post TBI using the most effective dose and time point as determined in AIM 2. Vehicle injection and sham operation will be used for controls. Beginning on the second day following TBI, animals will be tested for cognitive performance on a radial arm maze for 21 days.
Expected Results: We predict that Clazosentan will improve behavioral outcome.
Possible pitfalls, alternative approaches: In addition to the possibility of having to alter our dose of Clazosentan, we will also have screened our animals for motor deficits as this would likely confound our behavioral data. However, we have extensive experience in assessing behavioral outcome following TBI and have a battery of neurologic and cognitive tests available to us in the laboratory. To this end, it may be necessary to incorporate additional behavioral tests in order to accurately determine outcome. We are prepared to include Morris Water Maze tests if such a case arises.

GENERAL METHODS

Closed Head Trauma Model:
Adult male Sprague-Dawley rats (300-375g) (Harlan Industries) will be anesthetized with 5% halothane in 2% oxygen prior to intubation, and then maintained on 1.5% halothane via a mask and spontaneous breathing. Halothane will be used as the anesthetic for all experiments. The use of halothane instead of the more recently introduced isofluorane is preferred because of recent evidence indicating the latter neuroprotective properties (Zhao et al., 2007; Wei et al., 2007) and more recent data presented at the Brain '07, International Cerebral Blood Flow and Metabolism meeting held in Osaka, Japan. A midsagittal scalp incision will be performed and the underlying muscles retracted laterally. Cranioplastic cement will be used to attach a 10mm diameter X 3 mm thick, round metal helmet directly to the skill over the sagittal suture and between the coronal and lambdoidal sutures. The helmet is used to distribute the applied force over the surface of the parietal bones, thus preventing skull fractures with penetrating brain injury. After the cement is allowed to dry for three minutes, the animals will be placed prone on a platform as described in the Acceleration Impact Trauma Model of Marmarou (Marmarou et al., 1994). After 30-40 seconds of placement, 450g of weight contained in a hollow plastic cylinder will be dropped directly onto the helmet from a height of 2 meters. Brain and leg muscle temperatures will be taken routinely, in some instances up to 24 hrs post injury. We have determined that brain temperature fluctuated only 1.5°C, and muscle temperature 1.3°C, during this time period.

ET-1 ICV injections:

ICV injection will be performed on rats that will be anesthetized with IP injection of chloral hydrate (450 mg/kg; 4% solution) and placed in a small animal stereotaxic frame. A midline incision is made on the scalp to expose the skull. A sterile, 33-gauge steel guide cannula is positioned and affixed to the skull with dental cement to allow for ICV injections at the following coordinates referenced to Bregma: posterior -0.80 mm, lateral ±1.5 mm, ventral –3.8 mm (Paxinos and Watson, 2005). Bilateral stainless steel injectors (17mm) will be attached via Teflon tubing (gauge) to a 10μl Hamilton syringe placed in a Harvard microinjector. Vehicle (0.9% saline) or SU5416 dissolved in vehicle (10% dimethylsulphoxide (DSMO) will be injected bilaterally into the lateral ventricles over the course of 48- or 96 sec at a rate of 0.126 μl per minute for a total volume of 100 or 200 nanoliters.

Assessment of neuropathology:

FluoroJade: FluoroJade (FJ) will be performed as originally described (Schmued et al., 1997). Briefly 40 μm sections from smCx and hipp are washed in 80% ethanol with 1% NaOH, 70% ethanol and distilled water. Next tissue sections are washed in 0.06% $KmNO_4$ for 10 min and then washed in distilled water. FluoroJade solution (2 mL) is diluted in 48 mL 0.1% acetic acid and tissue is incubated for 30 min. Sections are then rinsed in distilled water, dehydrated, air dried and mounted in a water-based mounting media. Sections are analyzed as in the immunofluorescent techniques.

HSP-70 Immunocytochemistry: Serial sections (40 μm) of brain tissue containing the smCx and Hipp will be collected in multi-well plates and processed for HSP-70 immunocytochemistry. The sections will be washed in phosphate buffered saline (PBS) and treated in 80% formic acid for 10 min. Sections will then be washed and endogenous peroxidase activity blocked with 0.3% hydrogen peroxide in PBS for 1 hour then incubated overnight (1ug/ml) with a rabbit anti-HSP-70 (Zymed Laboratories). The antigen-antibody complex will be revealed using the avidin biotin peroxidase method using DAB as a substrate. The sections will be mounted onto glass slides with Permount and viewed with a Leica light microscope fixed with Axiocam software. Images of labeled with HSP-70 positive cells will be collected and quantified by manual counting and by optical densitometry.

TUNEL: TUNEL is performed on 40μm sections from smCx and Hipp using a commercially available In Situ Cell Death Detection Kit (Boehringer Mannheim, Brisbane, QLD, Australia). Briefly, tissue is fixed as before and mounted onto poly-l-lysine coated slides. Slides are then soaked in xylene (twice for 5 min) then ethanol (3 min in 96, 90, and 80%) before being rinsed in de-ionized water. Tissue is then permeabilized with proteinase K for 15 min at 37°C, rinsed with PBS and incubated for 60 min in the TUNEL mixture. After the incubation, sections are again rinsed in PBS, treated with alkaline phosphatase and then analyzed as in immunofluorescence. Quantification is accomplished by counting individual TUNEL-positive nuclei.

Caspase-3 Western Analysis: Whole brains will be harvested (n=4 animals per group), placed in cold methylbutane on dry ice and partially frozen. Brains will then be dissected to isolate smCx and Hipp. Isolated smCx and Hipp will be homogenized in Lamelli's solution and subjected to SDS-PAGE. Protein concentrations will be standardized for all samples. Electrophoresis will run at 40mA for the first 40 min and then 20mA for 3 hrs. Gels are transferred to nitrocellulose paper, and blocked using 1% nonfat dried milk in TTBS at room temperature for one hour. Transferred samples will then be incubated in primary antibody (mouse anti-Caspace-3, Sigma, St. Louis, MO) in TTBS at 4^0C overnight and then incubated in secondary antibody and donkey serum in TTBS at room temperature for 30 min. Nitrocellulose will be rinsed in Lumiglow solution for 90 seconds, exposed to X ray film and developed. Band intensity of immunoblots will be quantified using optical densitometric (OD) analysis.

DAI assessment and quantification:

DAI assessment: DAI will be assessed following TBI. The rats will be allowed to survive for 24 hrs, 48 hrs or one week. At the end of survival period, they will be sacrificed and transcardially perfused with normal saline followed by 4% paraformaldehyde. The brains will then be harvested and post fixed in the same perfusate for 24 hrs followed by cryoprotection with a solution containing 20% sucrose in 4% paraformaldehyde. Serial sections (40 μm) of brain tissue containing the smCx and Hipp will be collected in multi-well plates and processed for beta amyloid precursor protein (β-APP) immunocytochemistry. Briefly, the sections will be washed in phosphate buffered saline (PBS) and treated in 80% formic acid for 10 min. The sections will then be washed and endogenous peroxidase activity blocked with 0.3% hydrogen peroxide in PBS for 1 hour. The

sections will be then incubated overnight (1ug/ml) with a rabbit anti-APP C terminus antibody (Zymed Laboratories, CT 695). The antigen-antibody complex will be revealed using the avidin biotin peroxidase method using DAB as a substrate. The sections will be mounted onto glass slides with permount and coverslipped. The sections will be viewed with a Leica light microscope fixed with Axiocam software. Images of labeled retraction bulbs from Hipp and smCx will be collected and quantified. Manual counting of retraction bulbs and axon fragments in predetermined areas of white matter will be used to ultimately determine the extent of DAI.

Quantification using β-APP staining: Whole serial coronal sections (40μm thick) of brains immunostained for β-APP (as described above) will be selected. The corpus callosum will be selected for DAI analysis for two reasons: 1) previously documented DAI at 24 and 48 h post TBI using this model (Rafols et al., 2007), and 2) the nearly longitudinal arrangement of its axons in single coronal sections which facilitates tracing degenerating axons for relatively long distances. A series of six coronal sections through the entire anterior-posterior extent of corpus callosum will be selected from each brain at : 1) +2.04 mm; 2) +0.60 mm; 3) -0.84 mm; 4) -2.28 mm; 5) -3.00 mm and 6) -3.72 mm from bregma (coordinates after Paxinos and Watson, 2005). For DAI quantification, digital images (X10 objective lens) will be obtained of 7 different areas (area size=510X390□m) within CC from each of the above six levels (Figures 1A and 1B). In each of the seven areas, presence or absence of observed axon retraction bulbs and/or axonal swellings at single focal planes will be scored as either 1 or 0, respectively. A percentage incidence of DAI per brain will then be calculated by dividing the total observed DAI score per brain by 6(levels) X 42(areas). This semi-quantitative technique is based on a previously reported method (DiLeonardi et al., 2009). Extent of DAI between groups will then compared by One-Way ANOVA with least significant difference (LSD) post-hoc analysis using SPSS (Chicago, IL). A p value of <0.05 is considered significant.

Quantification using Diffuse Tensor Imaging (DTI) MRI: Diffusion tensor MR imaging (DTI) can be used to detect white matter pathology in experimental animal models of neurological diseases because the water diffusion is highly anisotropic in these tissues (see MacDonald et al., 1997 for review). It can provide information about brain microstructure by quantifying isotropic and anisotropic water diffusion. In white matter tracts, where most or all of the axons are aligned in a parallel fashion, diffusion parallel to the axons is greater than diffusion perpendicular to the axons. In contrast to DTI, conventional diffusion weighted imaging (DWI) uses only diffusivity averaged across all directions. Specifically, elements of the diffusion tensor may change in response to injury in such a way that average diffusivity changes very little. Diffusion can be evaluated by measuring the MR signal intensity attenuation as a function of the gradient factor or b-value.

All of the MRI measurements will be performed on a 4.7T horizontal-bore magnetic resonance spectrometer (AVANCE, Bruker, Karlsruhe, Germany) with an 11.6-cm bore actively shielded gradient coil set capable of producing a magnetic field gradient up to 250 mT/m. A whole-body birdcage radiofrequency (RF) coil (72mm inner diameter) will be used as the transmitter for homogeneous RF excitation and a surface coil (30mm diameter) as the receiver with active RF-decoupling to avoid signal interference.

Postprocessing of DTI images: DTI FA, ADC values and eigenvalues in both axial and two radial directions will be analyzed to identify the underlying pathology of FA decreases. The raw DTI data will be processed in DTI Studio v2.40 (http://cmrm.med.jhmi.edu/cmrm/DTIuser/DTIuser.html) to generate maps of FA, ADC, and three directional eigenvalues, all saved in analyze format files. The files will be opened in our in-house software SPIN for further quantitative analysis. For each ROI, we will assess the mean value and standard deviation of DTI parameters.

Assessment of motor deficit:
Since neurological deficits, while rarely seen using this model of TBI, would greatly hinder the ability of a rat to perform on the radial arm maze or in a Morris Water Maze, all animals will be screened following TBI for neurological outcome. In order to screen animals for motor deficit, all TBI animals will be tested using standard neurological function tests, including rotor rod performance, balance beam, and ladder climbing. Based on preliminary screens, rats either performed well or, on the contrary, showed deficit on all tests and, therefore, animals performing at sub-control levels on any test will be grounds for removal from the study.

Behavioral Testing and Radial Arm Maze Setup:
The rats will be allowed to acclimate to their new environment (in DLAR facility) after their arrival. Then from day 1 to day 3 of the behavioral study the rats will be handled by the researcher for 10 to 15 minutes each.

Acclimation to the maze environment also will be initiated during which the rats will be placed on the central platform of the radial arm maze and allowed to roam freely.

A custom designed radial arm maze will be built using black acrylic sheet (0.6 cm thick). Eight identical radial arms are fixed to an octagonal base platform that stands 63 cm above the floor. Each radial arm is 60 cm in length and 10 cm in width with 10 cm – high sidewalls along each arm. At the end of each arm a 5-cm end piece is placed. A hole measuring 2.5 cm in diameter is also cut 5 cm from the end of each radial arm to place a plastic food cup (1 oz).

During behavioral testing, the maze is enclosed within four black linen walls. A white paper triangle (15-cm sides) is placed on one linen wall 10 cm above the base of radial arm #3. An 8" x 11" white paper square with bisecting black lines is placed on the same linen wall 10 cm above the base of radial arm #5. A researcher holding a green notebook and always wearing a bright yellow surgical gown with latex gloves will be positioned in front of radial arm #8 during the study. These three visual cues are aimed to provide spatial guidance as to the location of the baited arm (i.e. containing the food).

Radial arm maze trials will commence from the 3rd day of the behavioral study. The rats will be tested for the time taken to find the bait (half of a Fruit Loop cereal®) placed in a plastic cup of four different radial arms. Each rat will be tested daily for three consecutive time trials. The maximum time a rat will be allowed to spend in the maze is ten minutes by the end of which determined to be conclusion of a trial. Averages of these trials will be calculated and recorded.

Statistical and Power Analysis:

Note: all statistical analyses will be conducted in accordance with Wayne State University's Biomedical Statistical Core facilities guidelines. Wayne State University's Core facility offers free consultation with a number of their staff at any time during the course of a funded project.

DTI assessment of DAI. A series of 6 scans will be conducted per animal and then averaged to determine an average FA per animal. Based on preliminary data, we have determined that 6 animals are needed per group to distinguish a difference in FA between groups with 90% power at $\alpha = 0.05$ using one-way analysis of variance (ANOVA) with LSD post hoc testing. Data are reported as mean ± SE. Significance is set at p-value < 0.05.

Western Analysis. All data pertaining to Western analysis of protein expression are expressed as the average of samples tested independently. Between group analyses are accomplished using ANOVA with LSD post-hoc testing. Data are reported as mean ± SE. Significance is set at p-value < 0.05. As previously reported (abstract in 2006 Society for Neuroscience Annual Meeting, Atlanta, GA; Kreipke 2007c), we were able to detect significant changes in protein expression between groups using 4 animals per group with 95% power at $\alpha = 0.05$.

Immunocytochemistry. All histological and immunocytochemical analyses are conducted using 4 to 6 sections per animal with 3 to 6 areas of analysis per section (see methodology). Data is expressed as an average of each area of analysis. In between group analysis is accomplished using ANOVA, with LSD post-hoc testing. Data are reported as mean ± SE. Significance is set at p-value < 0.05. Based on variability in these data from our previous studies (Kreipke et al., 2006, 2007a, b) we can distinguish a difference in individual proteins and in capillary density with 95% power at an α level of 0.05 with 4-6 rats per group.

Behavioral Assessments. All behavioral data are expressed as the average latency of completing the task over three trials. Between group analyses are accomplished using ANOVA with LSD post-hoc testing. Data are reported as mean ± SE. Significance is set at p-value < 0.05. Due to the variability in behavior amongst individual animals, we have previously determined (Kreipke 2004, 2007) that we can distinguish significant differences between TBI and control animals using 12 animals per group. Antagonist studies will require 12-15 animals per group to show an improved performance with power of 90%. This allows us to distinguish a difference in latency of 2 min with 90% power at $\alpha = 0.05$. Additional rats may be required due to failure to exercise, death, or motoric disability following injury.

TIME TABLE FOR EXPERIMENTS

Year I: determine effective dose of ET-1; determine effects of ET-1 on histopathology and behavior
Year 2: determine effects of ET-1 on DAI, determine effect of ETrA antagonism on DAI; write-up
Year 3: determine effects of ETrB antagonism on DAI, determine effect of ETrB on ET-1+TBI; write-up
Year 4: determine effects of Clazosentan on DAI; begin studies on effects of Clazosentan on behavior, write-up
Year 5: finish effects of Clazosentan on behavior; write-up; begin preliminary work for competing renewal

V. VERTEBRATE ANIMALS

The NIH-mandated five points regarding vertebrate animals are addressed as following:

1. **Provide a detailed description of the proposed use of the animals for the work outlined in the Research Design and Methods section. Identify the species, strains, ages, sex, and numbers of animals to be used in the proposed work.**

   For all experiments, Male Sprague-Dawley rats (400-450g) will be used. No more than 460 will be used in total.

2. **Justify the use of animals, the choice of species, and the numbers to be used. If animals are in short supply, costly, or to be used in large numbers, provide an additional rationale for their selection and numbers.**

   The choice to use male Sprague-Dawley rats is based on previous work both in our lab and in the labs cited in the research design. Due to careful use of animals for multiple experiments, no more than 460 male rats will be used in total. Further, rats will be used because of their low cost and because of the large body of information that is now known about their basic neuroanatomy, physiology, and behavior. Rats have an extremely high resistance to infection and are small in size which precludes using large amounts of expensive agents. In addition, the Sprague-Dawley strain has been shown to display pathological changes comparable to those encountered in clinical conditions.

3. **Provide information on the veterinary care of the animals involved.**

   Adherence to IACUC guidelines will be maintained in the experimental treatment and housing of the animals. Housing is provided in an IACUC approved facility in the same buildings as the laboratories (Dr. Rafols' laboratory and the Department of Animal Laboratory Research Testing Facility). Training in proper care and handling of animals, as provided by the Wayne State University Department of Laboratory Animal Resources, has been successfully completed by the applicant.

4. **Describe the procedures for ensuring that discomfort, distress, pain, and injury will be limited to that which is unavoidable in the conduct of scientifically sound research. Describe the use of analgesic, anesthetic, and tranquilizing drugs and/or comfortable restraining devices, where appropriate, to minimize discomfort, distress, pain, and injury.**

   After brain injury, some animals may experience persisting respiratory difficulties, and will be ventilated as necessary. If this ailment lasts longer than 60 min, such animals will be euthanized with sodium pentobarbital (120 mg/kg, IP injection) consistent with our previous work and with the Panel on Euthanasia of the American Veterinary Medical Association. It is possible that some degree of pain and distress will be present as a consequence of impact on the skull. However, animals are typically awake, but quiet and relatively inactive after trauma. By 1 hour they are usually active and are capable of eating and drinking on their own, although a drop of approximately 7% in body weight is expected. Analgesics will not be used immediately after injury because they (1) interfere with measurements of cerebrovascular function, (2) have neuroprotective effects and (3) in our experience with humans, there is very little or no need for analgesics right after a severe head injury. The effects of analgesics would compromise the results from the proposed experiments.

5. **Describe any method of euthanasia to be used and the reason(s) for its selection. State whether this method is consistent with the recommendations of the American Veterinary**

Medical Association (AVMA) Guidelines on Euthanasia. If not, include a scientific justification for not following the recommendations.

Upon termination of a given testing period, rats will be euthanized with a lethal dose of sodium pentobarbital (120 mg/kg IP as above) and death will be assured by bilateral pneumothorax and severing the aorta.

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PHS 398 Checklist

OMB Number: 0925-0001

1. Application Type:

From SF 424 (R&R) Cover Page. The responses provided on the R&R cover page are repeated here for your reference, as you answer the questions that are specific to the PHS398.

* Type of Application:

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

Federal Identifier:

2. Change of Investigator / Change of Institution Questions

☐ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:
* First Name:
Middle Name:
* Last Name:
Suffix:

☐ Change of Grantee Institution

* Name of former institution:

3. Inventions and Patents (For renewal applications only)

* Inventions and Patents: Yes ☐ No ☐

If the answer is "Yes" then please answer the following:

* Previously Reported: Yes ☐ No ☐

4. * Program Income

Is program income anticipated during the periods for which the grant support is requested?

☐ Yes ☒ No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

5. * Disclosure Permission Statement

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

☒ Yes ☐ No